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(57) Abstract

The invention relates to relatively short peptides (termed τ–conotoxins herein), about 10–25 residues in length, which are naturally available in minute amounts in the venom of the cone snails or analogous to the naturally available peptides, and which preferably include two disulfide bonds.

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TITLE OF THE INVENTION

TAU-CONOTOXIN PEPTIDES

This invention was made with Government support under Grant No. PO1 GM48677 awarded by the National Institute of General Medical Sciences, National Institutes of Health, Bethesda, Maryland. The United States Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The invention relates to relatively short peptides (termed τ-conotoxins herein), about 10-20 residues in length, which are naturally available in minute amounts in the venom of the cone snails or analogous to the naturally available peptides, and which preferably include two disulfide bonds.

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference, and for convenience are referenced in the following text by author and date and are listed alphabetically by author in the appended bibliography.

The predatory cone snails (*Conus*) have developed a unique biological strategy. Their venom contains relatively small peptides that are targeted to various neuromuscular receptors and may be equivalent in their pharmacological diversity to the alkaloids of plants or secondary metabolites of microorganisms. Many of these peptides are among the smallest nucleic acid-encoded translation products having defined conformations, and as such, they are somewhat unusual. Peptides in this size range normally equilibrate among many conformations. Proteins having a fixed conformation are generally much larger.

The cone snails that produce these peptides are a large genus of venomous gastropods comprising approximately 500 species. All cone snail species are predators that inject venom to capture prey, and the spectrum of animals that the genus as a whole can envenomate is broad. A wide variety of hunting strategies are used, however, every *Conus* species uses fundamentally the same basic pattern of envenomation.

Several peptides isolated from *Conus* venoms have been characterized. These include the α-, μ- and ω-conotoxins which target nicotinic acetylcholine receptors, muscle sodium channels,

and neuronal calcium channels, respectively (Olivera et al., 1985). Conopressins, which are vasopressin analogs, have also been identified (Cruz et al.. 1987). In addition, peptides named conantokins have been isolated from *Conus geographus* and *Conus tulipa* (Mena et al., 1990; Haack et al., 1990).

Chronic or intractable pain, which may result from degenerative conditions or debilitating diseases, is currently treated with a variety of analgesic compounds, often opioid compounds such as morphine. Likewise, neuropathic pain, typically a chronic condition attributable to injury or partial transection of a peripheral nerve, is also conventionally treated with opioid compounds such as morphine.

Conventional therapies for pain produce analgesia--a loss of sensitivity to pain without the loss of consciousness. Opioid compounds have been used widely to produce analgesia, including plant-derived opioids such as morphine, and endogenous opioids such as met- and leu-enkephalins, as well as beta-endorphin.

Opioid compounds, while effective in producing analgesia for many types of pain, may induce tolerance in some patients. When a patient becomes tolerant, increasing doses of the opioid are required to produce the desired analgesic effect. In addition, these compounds frequently result in a physical dependence in patients, and may have side effects at high doses.

The analgesic effects and adverse actions of various NMDA receptor antagonists has been shown to vary depending on the site of action and potency of the drug. For example, NMDA receptor antagonists acting at the ion channel in a noncompetitive manner (e.g., MK-801 and phenylcyclidine (PCP)) or competitive inhibitors, show analgesic activity but show motor impairment at equivalent doses. Glycine B-site NMDA antagonists appear to have analgesic activity at doses that do not impair motor function. Conantokins, which are polyamine-site NMDA antagonist compounds have analgesic effects at doses which do not produce overt side effects (PCT published application WO 98/03189).

It is desired to provide additional compounds which have analgesic properties.

SUMMARY OF THE INVENTION

The invention relates to relatively short peptides (termed τ-conotoxins herein), about 10-25 residues in length, which are naturally available in minute amounts in the venom of the cone snails or analogous to the naturally available peptides, and which preferably include two disulfide bonds.

More specifically, the present invention is directed to τ-conotoxin peptides having the general formula I:

Xaa_1-Xaa_2-Xaa_3-Xaa_4-Cys-Cys-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Cys-Cys-Xaa_{10}-Xaa_{11}-Xaa_{12}-Xaa_{13}-Xaa_{14}-Xaa_{15}-Xaa_{16}-Xaa_{17}-Xaa_{18}-Xaa_{19} (SEQ ID NO:1), wherein Xaa_1 is des-Xaa_1, Asp, Glu or γ-carboxy-Glu (Gla); Xaa_2 is des-Xaa_2, Gln, Asn, Glu, Trp (D or L), neo-Trp, halo-Trp or any unnatural aromatic amino acid; Xaa_3 is des-Xaa_3, Gly, Ala, Asn or Gln; Xaa_4 is des-Xaa_4,Val, Leu (D or L), Ile, Ala, Gly, Glu, Gla, Asp, Ser, Thr, Phe, Trp (D or L), neo-Trp, halo-Trp (D or L) or any unnatural aromatic amino acid; Xaa_5 is Pro, hydroxy-Pro, Gln, Asn, Glu, Gla, Ala, Gly, Lys, Arg, Ile, Val, homoarginine, ornithine, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; Xaa_6 is Val, Phe, Thr, Ser, Glu, Gla, Asp, Asn, Gln, Ala, Gly, Ile, Leu (D or L), Met, Pro, hydroxy-Pro, Arg, homoarginine, ornithine, Lys, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa_7 is any Val, Ile, Asn, Leu (D or L), Gln, Gly, Ala, Phe, Glu, Gla, Arg, ornithine, homoarginine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa_8 is Ile, Leu (D or L), Met, Thr, Ser, Pro, hydroxy-Pro, Gln, Asp, Glu, Gla, Asn, Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr, nitro-Tyr, any unnatural basic amino acid, any unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa_9 is des-Xaa_9, Ala, Gly, Asp, Glu, Gla, Trp (D or L) neo-Trp, halo-Trp (D or L), Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Arg, homoarginine, ornithine, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr, nitro-Tyr or any unnatural basic amino acid; Xaa_{10} is des-Xaa_{10}, Ile, Leu (D or L), Val, Glu, Gla, Asp, Thr, Ser, Pro, hydroxy-Pro, Trp (D or L), neo-Trp, halo-Trp (D or L), Phe, any unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa_{11} is des-Xaa_{11}, Gln, Asn, Leu (D or L), Ile, Val, Ala, Gly, Trp (D or L), neo-Trp, halo-Trp (D or L), Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa_{12} is des-Xaa_{12}, Ala, Gly, Phe, Trp (D or L), neo-Trp, halo-Trp (D or L) or any unnatural aromatic amino acid; Xaa_{13} is des-Xaa_{13}, Glu, Gla, Asp, Phe or any unnatural aromatic amino acid; Xaa_{14} is des-Xaa_{14}, Ile, Val or Leu (D or L); Xaa_{15} is des-Xaa_{15}, Thr, Ser, Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; Xaa_{16} is des-Xaa_{16}, Glu, Gla or Asp; Xaa_{17} is des-Xaa_{17}, Asn or Gln; Xaa_{18} is des-Xaa_{18}, Asp, Glu or Gla; Xaa_{19} is des-Xaa_{19}, Phe or any unnatural aromatic amino

acid. The C-terminus may contain a free carboxyl group or an amide group. The halo is preferably bromine, chlorine or iodine, more preferably iodine for Tyr and bromine for Trp. The Cys residues may be in D or L configuration and may optionally be substituted with homocysteine (D or L). The Tyr residues may be substituted with the 3-hydroxyl or 2-hydroxyl isomers and corresponding O-sulpho- and O-phospho-derivatives. The acidic amino acid residues may be substituted with any synthetic acidic amino acid, e.g., tetrazolyl derivatives of Gly and Ala.

The present invention is also directed to novel specific τ-conotoxin peptides of general formula I having the formulas:

Phe-Cys-Cys-Xaa_1-Val-Ile-Arg-Xaa_2-Cys-Cys-Xaa_3 (SEQ ID NO:2);

Phe-Cys-Cys-Xaa_1-Phe-Ile-Arg-Xaa_2-Cys-Cys-Xaa_3 (SEQ ID NO:3);

Cys-Cys-Gln-Thr-Phe-Xaa_2-Xaa_3-Cys-Cys-Gln (SEQ ID NO:4);

Xaa_4-Gly-Xaa_3-Cys-Cys-Xaa_5-Xaa_6-Asn-Ile-Ala-Cys-Cys-Ile (SEQ ID NO:5);

Gly-Cys-Cys-Ala-Arg-Leu-Thr-Cys-Cys-Val (SEQ ID NO:6);

Asn-Gly-Cys-Cys-Xaa_1-Xaa_5-Gln-Met-Arg-Cys-Cys-Thr (SEQ ID NO:7);

Asp-Xaa_3-Asn-Ser-Cys-Cys-Gly-Xaa_5-Asn-Xaa_1-Gly-Cys-Cys-Xaa_1-Xaa_3 (SEQ ID NO:8);

Xaa_4-Gly-Xaa_3-Cys-Cys-Xaa_5-Xaa_6-Asn-Ile-Arg-Cys-Cys-Val (SEQ ID NO:9);

Xaa_6-Cys-Cys-Xaa_6-Asp-Gly-Xaa_3-Cys-Cys-Thr-Ala-Ala-Xaa_1-Leu-Thr (SEQ ID NO:10);

Gly-Cys-Cys-Xaa_6-Asp-Gly-Xaa_3-Cys-Cys-Thr-Ala-Ala-Xaa_1-Leu-Thr(SEQ ID NO:11);

Asn-Gly-Cys-Cys-Arg-Ala-Gly-Asp-Cys-Cys-Ser-Arg-Phe-Xaa_6-Ile-Xaa_5-Xaa_6-Asn-Asp-Phe (SEQ ID NO:12);

Asn-Ala-Cys-Cys-Ile-Val-Arg-Gln-Cys-Cys (SEQ ID NO:13);

Asn-Gly-Cys-Cys-Arg-Ala-Gly-Asp-Cys-Cys-Ser (SEQ ID NO:14);

Cys-Cys-Xaa_1-Arg-Arg-Leu-Ala-Cys-Cys-Ile-Ile (SEQ ID NO:15);

Cys-Cys-Xaa_1-Asn-Xaa_5-Xaa_1-Cys-Cys-Phe-Ile (SEQ ID NO:16);

Gly-Cys-Cys-Ala-Met-Leu-Thr-Cys-Cys-Val (SEQ ID NO:17);

Leu-Cys-Cys-Val-Thr-Xaa_6-Asp-Xaa_3-Cys-Cys-Xaa_6-Xaa_3-Xaa_3 (SEQ ID NO:18); and

Val-Cys-Cys-Arg-Xaa_1-Val-Gln-Asp-Cys-Cys-Ser (SEQ ID NO:19);

wherein Xaa_1 is Pro or hydroxy-Pro; Xaa_2 is Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr or nitro-Tyr; Xaa_3 is Trp or halo-Trp; Xaa_4 is Gln or pyro-Glu; Xaa_5 is Lys, N-methyl-Lys, N,N-dimethyl-Lys or N,n,N-trimethyl-Lys, Xaa_6 is Glu or gamma-carboxy-Glu (Gla); and the C-terminus contains a carboxyl or amide group. The halo is preferably bromine, chlorine or iodine, more preferably iodine for Tyr and bromine for Trp. In addition, the Arg residues may be

substituted by Lys, ornithine, homoargine, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; the Lys residues may be substituted by Arg, ornithine, homoargine, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; the Tyr residues may be substituted with any unnatural hydroxy containing amino acid; the Ser residues may be substituted with Thr; the Thr residues may be substituted with Ser; and the Phe and Trp residues may be substituted with any unnatural aromatic amino acid. The Cys residues may be in D or L configuration and may optionally be substituted with homocysteine (D or L). The Tyr residues may be substituted with the 3-hydroxyl or 2-hydroxyl isomers and corresponding O-sulpho- and O-phospho-derivatives. The acidic amino acid residues may be substituted with any synthetic acidic amino acid, e.g., tetrazolyl derivatives of Gly and Ala.

More specifically, the present invention is directed to the following τ-conotoxin peptides of general formula I:

AuVA:	SEQ ID NO:2, wherein Xaa_1 is Pro, Xaa_2 is Tyr and Xaa_3 is Trp;
AuVB:	SEQ ID NO:3, wherein Xaa_1 is Pro, Xaa_2 is Tyr and Xaa_3 is Trp;
Tx5.1:	SEQ ID NO:4, wherein Xaa_2 is Tyr and Xaa_3 is Trp;
G5.1:	SEQ ID NO:5, wherein Xaa_3 is Trp, Xaa_4 is Gln, Xaa_5 is Lys and Xaa_6 is Glu;
Qc5.1:	SEQ ID NO:6;
PVA:	SEQ ID NO:7, wherein Xaa_1 is Pro and Xaa_5 is Lys;
Im5.1:	SEQ ID NO:8, wherein Xaa_1 is Pro, Xaa_3 is Trp and Xaa_5 is Lys;
G5.2:	SEQ ID NO:9, wherein Xaa_3 is Trp, Xaa_4 is Gln, Xaa_5 is Lys and Xaa_6 is Glu;
Tx5.2a:	SEQ ID NO:10, wherein Xaa_1 is Pro, Xaa_3 is Trp and Xaa_6 is Glu;
Tx5.2b:	SEQ ID NO:11, wherein Xaa_1 is Pro, Xaa_3 is Trp and Xaa_6 is Glu;
Mr5.1:	SEQ ID NO:12, wherein Xaa_5 is Lys and Xaa_6 is Glu;
Mr5.2:	SEQ ID NO:13;
Mr5.3:	SEQ ID NO:14;
Ca5.1:	SEQ ID NO:15, wherein Xaa_1 is Pro;
Ca5.2:	SEQ ID NO:16, wherein Xaa_1 is Pro and Xaa_5 is Lys;
Qc5.2:	SEQ ID NO:17;
Gm5.1:	SEQ ID NO:18, wherein Xaa_3 is Trp and Xaa_6 is Glu; and
Gm5.2:	SEQ ID NO:19, wherein Xaa_1 is Pro.

The C-terminus preferably contains a carboxyl group for the peptides AuVA, AuVB, G5.1, PVA, G5.2, Mr5.2, Mr5.3 and Gm5.1 The C-terminus of the other peptides preferably contains an amide group.

Examples of unnatural aromatic amino acid include, but are not limited to, such as nitro-Phe, 4-substituted-Phe wherein the substituent is C_1-C_3 alkyl, carboxyl, hyrdroxymethyl, sulphomethyl, halo, phenyl, -CHO, -CN, -SO_3H and -NHAc. Examples of unnatural hydroxy containing amino acid, include, but are not limited to, such as 4-hydroxymethyl-Phe, 4-hydroxyphenyl-Gly, 2,6-dimethyl-Tyr and 5-amino-Tyr. Examples of unnatural basic amino acids include, but are not limited to, N-1-(2-pyrazolinyl)-Arg, 2-(4-piperinyl)-Gly, 2-(4-piperinyl)-Ala, 2-[3-(2S)pyrrolininyl)-Gly and 2-[3-(2S)pyrrolininyl)-Ala. These and other unnatural basic amino acids, unnatural hydroxy containing amino acids or unnatural aromatic amino acids are described in Building Block Index, Version 3.0 (1999 Catalog, pages 4-47 for hydroxy containing amino acids and aromatic amino acids and pages 66-87 for basic amino acids; see also http://www.amino-acids.com), incorporated herein by reference, by and available from RSP Amino Acid Analogues, Inc., Worcester, MA. Examples of synthetic acid amino acids include those derivatives bearing acidic functionality, including carboxyl, phosphate, sulfonate and synthetic tetrazolyl derivatives such as described by Ornstein et al. (1993) and in U.S. Patent No. 5,331,001, each incorporated herein by reference.

Optionally, in the peptides of general formula I and the specific peptides described above, the Asn residues may be modified to contain an N-glycan and the Ser and Thr residues may be modified to contain an O-glycan. In accordance with the present invention, a glycan shall mean any N-, S- or O-linked mono-, di-, tri-, poly- or oligosaccharide that can be attached to any hydroxy, amino or thiol group of natural or modified amino acids by synthetic or enzymatic methodologies known in the art. The monosaccharides making up the glycan can include D-allose, D-altrose, D-glucose, D-mannose, D-gulose, D-idose, D-galactose, D-talose, D-galactosamine, D-glucosamine, D-N-acetyl-glucosamine (GlcNAc), D-N-acetyl-galactosamine (GalNAc), D-fucose or D-arabinose. These saccharides may be structurally modified, e.g., with one or more O-sulfate, O-phosphate, O-acetyl or acidic groups, such as sialic acid, including combinations thereof. The gylcan may also include similar polyhydroxy groups, such as D-penicillamine 2,5 and halogenated derivatives thereof or polypropylene glycol derivatives. The glycosidic linkage is beta and 1-4 or 1-3, preferably 1-3. The linkage between the glycan and the amino acid may be alpha or beta, preferably alpha and is 1-.

Core O-glycans have been described by Van de Steen et al. (1998), incorporated herein by reference. Mucin type O-linked oligosaccharides are attached to Ser or Thr (or other hydroxylated residues of the present peptides) by a GalNAc residue. The monosaccharide building blocks and the linkage attached to this first GalNAc residue define the "core glycans," of which eight have been identified. The type of glycosidic linkage (orientation and connectivities) are defined for each core glycan. Suitable glycans and glycan analogs are described further in U.S. Serial No. 09/420,797, filed 19 October 1999 and in PCT Application No. PCT/US99/24380, filed 19 October 1999, both incorporated herein by reference. A preferred glycan is Gal(β1→3)GalNAc(α1→).

Optionally, in the peptides of general formulas I and II and the specific peptides described above, pairs of Cys residues may be replaced pairwise with isoteric lactam or ester-thioether replacements, such as Ser/(Glu or Asp), Lys/(Glu or Asp) or Cys/Ala combinations. Sequential coupling by known methods (Barnay et al., 2000; Hruby et al., 1994; Bitan et al., 1997) allows replacement of native Cys bridges with lactam bridges. Thioether analogs may be readily synthesized using halo-Ala residues commercially available from RSP Amino Acid Analogues.

The present invention is further directed to propeptides and nucleic acid sequences encoding the propeptides or peptides as described in further detail herein.

SUMMARY OF THE SEQUENCE LISTING

SEQ ID NO:1 is generic formula I for τ-conotoxin peptides. SEQ ID NO:2 is a generic formula for the peptide AuVA. SEQ ID NO:3 is a generic formula for the peptide AuVB. SEQ ID NO:4 is a generic formula for the peptide Tx5.1. SEQ ID NO:5 is a generic formula for the peptide G5.1. SEQ ID NO:6 is a generic formula for the peptide Qc5.1. SEQ ID NO:7 is a generic formula for the peptide PVA. SEQ ID NO:8 is a generic formula for the peptide Im5.1. SEQ ID NO:9 is a generic sequence for the peptide G5.2. SEQ ID NO:10 is a generic sequence for the peptide Tx5.2a. SEQ ID NO:11 is a generic sequence for the peptide Tx5.2b. SEQ ID NO:12 is a generic sequence for the peptide Mr5.1. SEQ ID NO:13 is a generic sequence for the peptide Mr5.2. SEQ ID NO:14 is a generic formula for the peptide Mr5.3. SEQ ID NO:15 is a generic formula for the peptide Ca5.1. SEQ ID NO:16 is a generic formula for the peptide Ca5.2. SEQ ID NO:17 is a generic formula for the peptide Qc5.2. SEQ ID NO:18 is a generic formula for the peptide Gm5.1. SEQ ID NO:19 is a generic formula for the peptide Gm5.2. SEQ ID NO:20 is a DNA sequence coding for the Tx5.1 propeptide. SEQ ID NO:21 is the amino acid sequence of the Tx5.1 propeptide. SEQ ID NO:22 is a DNA sequence coding for the G5.1 propeptide. SEQ ID NO:23

is the amino acid sequence of the G5.1 propeptide. SEQ ID NO:24 is a DNA sequence coding for the Qc5.1 propeptide. SEQ ID NO:25 is the amino acid sequence of the Qc5.1 propeptide. SEQ ID NO:26 is a DNA sequence coding for the Im5.1 propeptide. SEQ ID NO:27 is the amino acid sequence of the Im5.1 propeptide. SEQ ID NO:28 is a DNA sequence coding for the G5.2 propeptide. SEQ ID NO:29 is the amino acid sequence of the G5.2 propeptide. SEQ ID NO:30 is a DNA sequence coding for the Tx5.2 propeptide. SEQ ID NO:31 is the amino acid sequence of the Tx5.2 propeptide. SEQ ID NO:32 is a DNA sequence coding for the Tx5.3 propeptide. SEQ ID NO:33 is the amino acid sequence of the Tx5.3 propeptide. SEQ ID NO:34 is a DNA sequence coding for the Mr5.1 peptide. SEQ ID NO:35 is the amino acid sequence of the Mr5.1 peptide. SEQ ID NO:36 is a DNA sequence coding for the Mr5.2 peptide. SEQ ID NO:37 is the amino acid sequence of the Mr5.2 peptide. SEQ ID NO:38 is a DNA sequence coding for the Mr5.3 propeptide. SEQ ID NO:39 is the amino acid sequence of the Mr5.3 propeptide. SEQ ID NO:40 is a DNA sequence coding for the Ca5.1 propeptide. SEQ ID NO:41 is the amino acid sequence of the Ca5.1 propeptide. SEQ ID NO:42 is a DNA sequence coding for the Ca5.2 propeptide. SEQ ID NO:43 is the amino acid sequence of the Ca5.2 propeptide. SEQ ID NO:44 is a DNA sequence coding for the Qc5.2 propeptide. SEQ ID NO:45 is the amino acid sequence of the Qc5.2 propeptide. SEQ ID NO:46 is a DNA sequence coding for the Gm5.1 propeptide. SEQ ID NO:47 is the amino acid sequence of the Gm5.1 propeptide. SEQ ID NO:48 is a DNA sequence coding for the Gm5.2 propeptide. SEQ ID NO:49 is the amino acid sequence of the Gm5.2 propeptide.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The invention relates to relatively short peptides (termed τ-conotoxins herein), about 10-25 residues in length, which are naturally available in minute amounts in the venom of the cone snails or analogous to the naturally available peptides, and which preferably include two disulfide bonds.

The present invention, in another aspect, relates to a pharmaceutical composition comprising an effective amount of an τ-conotoxin peptide, a mutein thereof, an analog thereof, an active fragment thereof or pharmaceutically acceptable salts . Such a pharmaceutical composition has the capability of acting as an antagonist for acetylcholine receptors and as analgesic agents for the treatment of pain, including migraine. Thus, the pharmaceutical compositions of the present invention are useful in the treatment of pain (whether acute or chronic), including chronic pain, and neuropathic pain, without undesirable side effects.

The τ-conotoxin peptides described herein are sufficiently small to be chemically synthesized. General chemical syntheses for preparing the foregoing τ-conotoxin peptides are described hereinafter. Various ones of the τ-conotoxin peptides can also be obtained by isolation and purification from specific *Conus* species using the technique described in U.S. Patent Nos. 4,447,356 (Olivera et al., 1984); 5,514,774; 5,719,264; and 5,591,821, as well as in PCT published application WO 98/03189, the disclosures of which are incorporated herein by reference.

Although the τ-conotoxin peptides of the present invention can be obtained by purification from cone snails, because the amounts of τ-conotoxin peptides obtainable from individual snails are very small, the desired substantially pure τ-conotoxin peptides are best practically obtained in commercially valuable amounts by chemical synthesis using solid-phase strategy. For example, the yield from a single cone snail may be about 10 micrograms or less of τ-conotoxin peptide. By "substantially pure" is meant that the peptide is present in the substantial absence of other biological molecules of the same type; it is preferably present in an amount of at least about 85% purity and preferably at least about 95% purity. Chemical synthesis of biologically active τ-conotoxin peptides depends of course upon correct determination of the amino acid sequence.

The τ-conotoxin peptides can also be produced by recombinant DNA techniques well known in the art. Such techniques are described by Sambrook et al. (1989). A gene of interest (i.e., a gene that encodes a suitable τ-conotoxin peptide) can be inserted into a cloning site of a suitable expression vector by using standard techniques. These techniques are well known to those skilled in the art. The expression vector containing the gene of interest may then be used to transfect the desired cell line. Standard transfection techniques such as calcium phosphate co-precipitation, DEAE-dextran transfection or electroporation may be utilized. A wide variety of host/expression vector combinations may be used to express a gene encoding a conotoxin peptide of interest. Such combinations are well known to a skilled artisan. The peptides produced in this manner are isolated, reduced if necessary, and oxidized to form the correct disulfide bonds.

One method of forming disulfide bonds in the τ-conotoxin peptides of the present invention is the air oxidation of the linear peptides for prolonged periods under cold room temperatures or at room temperature. This procedure results in the creation of a substantial amount of the bioactive, disulfide-linked peptides. The oxidized peptides are fractionated using reverse-phase high performance liquid chromatography (HPLC) or the like, to separate peptides having different linked configurations. Thereafter, either by comparing these fractions with the elution of the native material or by using a simple assay, the particular fraction having the correct linkage for maximum biological

potency is easily determined. However, because of the dilution resulting from the presence of other fractions of less biopotency, a somewhat higher dosage may be required.

The peptides are synthesized by a suitable method, such as by exclusively solid-phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution couplings.

In conventional solution phase peptide synthesis, the peptide chain can be prepared by a series of coupling reactions in which constituent amino acids are added to the growing peptide chain in the desired sequence. Use of various coupling reagents, e.g., dicyclohexylcarbodiimide or diisopropylcarbonyldimidazole, various active esters, e.g., esters of N-hydroxyphthalimide or N-hydroxy-succinimide, and the various cleavage reagents, to carry out reaction in solution, with subsequent isolation and purification of intermediates, is well known classical peptide methodology. Classical solution synthesis is described in detail in the treatise, "Methoden der Organischen Chemie (Houben-Weyl): Synthese von Peptiden," (1974). Techniques of exclusively solid-phase synthesis are set forth in the textbook, "Solid-Phase Peptide Synthesis," (Stewart and Young, 1969), and are exemplified by the disclosure of U.S. Patent 4,105,603 (Vale et al., 1978). The fragment condensation method of synthesis is exemplified in U.S. Patent 3,972,859 (1976). Other available syntheses are exemplified by U.S. Patents No. 3,842,067 (1974) and 3,862,925 (1975). The synthesis of peptides containing γ-carboxyglutamic acid residues is exemplified by Rivier et al. (1987), Nishiuchi et al. (1993) and Zhou et al. (1996).

Common to such chemical syntheses is the protection of the labile side chain groups of the various amino acid moieties with suitable protecting groups which will prevent a chemical reaction from occurring at that site until the group is ultimately removed. Usually also common is the protection of an α-amino group on an amino acid or a fragment while that entity reacts at the carboxyl group, followed by the selective removal of the α-amino protecting group to allow subsequent reaction to take place at that location. Accordingly, it is common that, as a step in such a synthesis, an intermediate compound is produced which includes each of the amino acid residues located in its desired sequence in the peptide chain with appropriate side-chain protecting groups linked to various ones of the residues having labile side chains.

As far as the selection of a side chain amino protecting group is concerned, generally one is chosen which is not removed during deprotection of the α-amino groups during the synthesis. However, for some amino acids, e.g., His, protection is not generally necessary. In selecting a particular side chain protecting group to be used in the synthesis of the peptides, the following

general rules are followed: (a) the protecting group preferably retains its protecting properties and is not split off under coupling conditions, (b) the protecting group should be stable under the reaction conditions selected for removing the α-amino protecting group at each step of the synthesis, and (c) the side chain protecting group must be removable, upon the completion of the synthesis containing the desired amino acid sequence, under reaction conditions that will not undesirably alter the peptide chain.

It should be possible to prepare many, or even all, of these peptides using recombinant DNA technology. However, when peptides are not so prepared, they are preferably prepared using the Merrifield solid-phase synthesis, although other equivalent chemical syntheses known in the art can also be used as previously mentioned. Solid-phase synthesis is commenced from the C-terminus of the peptide by coupling a protected α-amino acid to a suitable resin. Such a starting material can be prepared by attaching an α-amino-protected amino acid by an ester linkage to a chloromethylated resin or a hydroxymethyl resin, or by an amide bond to a benzhydrylamine (BHA) resin or para-methylbenzhydrylamine (MBHA) resin. Preparation of the hydroxymethyl resin is described by Bodansky et al. (1966). Chloromethylated resins are commercially available from Bio Rad Laboratories (Richmond, CA) and from Lab. Systems, Inc. The preparation of such a resin is described by Stewart and Young (1969). BHA and MBHA resin supports are commercially available, and are generally used when the desired polypeptide being synthesized has an unsubstituted amide at the C-terminus. Thus, solid resin supports may be any of those known in the art, such as one having the formulae -O-CH_2-resin support, -NH BHA resin support, or -NH-MBHA resin support. When the unsubstituted amide is desired, use of a BHA or MBHA resin is preferred, because cleavage directly gives the amide. In case the N-methyl amide is desired, it can be generated from an N-methyl BHA resin. Should other substituted amides be desired, the teaching of U.S. Patent No. 4,569,967 (Kornreich et al., 1986) can be used, or should still other groups than the free acid be desired at the C-terminus, it may be preferable to synthesize the peptide using classical methods as set forth in the Houben-Weyl text (1974).

The C-terminal amino acid, protected by Boc or Fmoc and by a side-chain protecting group, if appropriate, can be first coupled to a chloromethylated resin according to the procedure set forth in K. Horiki et al. (1978), using KF in DMF at about 60°C for 24 hours with stirring, when a peptide having free acid at the C-terminus is to be synthesized. Following the coupling of the BOC-protected amino acid to the resin support, the α-amino protecting group is removed, as by using trifluoroacetic acid (TFA) in methylene chloride or TFA alone. The deprotection is carried out at

a temperature between about 0°C and room temperature. Other standard cleaving reagents, such as HCl in dioxane, and conditions for removal of specific α-amino protecting groups may be used as described in Schroder & Lubke (1965).

After removal of the α-amino-protecting group, the remaining α-amino- and side chain-protected amino acids are coupled step-wise in the desired order to obtain the intermediate compound defined hereinbefore, or as an alternative to adding each amino acid separately in the synthesis, some of them may be coupled to one another prior to addition to the solid phase reactor. Selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable as a coupling reagent is N,N'-dicyclohexylcarbodiimide (DCC, DIC, HBTU, HATU, TBTU in the presence of HoBt or HoAt).

The activating reagents used in the solid phase synthesis of the peptides are well known in the peptide art. Examples of suitable activating reagents are carbodiimides, such as N,N'-diisopropylcarbodiimide and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide. Other activating reagents and their use in peptide coupling are described by Schroder & Lubke (1965) and Kapoor (1970).

Each protected amino acid or amino acid sequence is introduced into the solid-phase reactor in about a twofold or more excess, and the coupling may be carried out in a medium of dimethylformamide (DMF):CH_2Cl_2 (1:1) or in DMF or CH_2Cl_2 alone. In cases where intermediate coupling occurs, the coupling procedure is repeated before removal of the α-amino protecting group prior to the coupling of the next amino acid. The success of the coupling reaction at each stage of the synthesis, if performed manually, is preferably monitored by the ninhydrin reaction, as described by Kaiser et al. (1970). Coupling reactions can be performed automatically, as on a Beckman 990 automatic synthesizer, using a program such as that reported in Rivier et al. (1978).

After the desired amino acid sequence has been completed, the intermediate peptide can be removed from the resin support by treatment with a reagent, such as liquid hydrogen fluoride or TFA (if using Fmoc chemistry), which not only cleaves the peptide from the resin but also cleaves all remaining side chain protecting groups and also the α-amino protecting group at the N-terminus if it was not previously removed to obtain the peptide in the form of the free acid. If Met is present in the sequence, the Boc protecting group is preferably first removed using trifluoroacetic acid (TFA)/ethanedithiol prior to cleaving the peptide from the resin with HF to eliminate potential S-alkylation. When using hydrogen fluoride or TFA for cleaving, one or more scavengers such as anisole, cresol, dimethyl sulfide and methylethyl sulfide are included in the reaction vessel.

Cyclization of the linear peptide is preferably affected, as opposed to cyclizing the peptide while a part of the peptido-resin, to create bonds between Cys residues. To effect such a disulfide cyclizing linkage, fully protected peptide can be cleaved from a hydroxymethylated resin or a chloromethylated resin support by ammonolysis, as is well known in the art, to yield the fully protected amide intermediate, which is thereafter suitably cyclized and deprotected. Alternatively, deprotection, as well as cleavage of the peptide from the above resins or a benzhydrylamine (BHA) resin or a methylbenzhydrylamine (MBHA), can take place at 0°C with hydrofluoric acid (HF) or TFA, followed by oxidation as described above.

The peptides are also synthesized using an automatic synthesizer. Amino acids are sequentially coupled to an MBHA Rink resin (typically 100 mg of resin) beginning at the C-terminus using an Advanced Chemtech 357 Automatic Peptide Synthesizer. Couplings are carried out using 1,3-diisopropylcarbodimide in N-methylpyrrolidinone (NMP) or by 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and diethylisopro- pylethylamine (DIEA). The FMOC protecting group is removed by treatment with a 20% solution of piperidine in dimethylformamide(DMF). Resins are subsequently washed with DMF (twice), followed by methanol and NMP.

Muteins, analogs or active fragments, of the foregoing τconotoxin peptides are also contemplated here. See, e.g., Hammerland et al, Eur. J. Pharmacol., 226, pp. 239-244 (1992). Derivative muteins, analogs or active fragments of the conotoxin peptides may be synthesized according to known techniques, including conservative amino acid substitutions, such as outlined in U.S. Pat. Nos. 5,545,723 (see particularly col. 2, line 50--col. 3, line 8); 5,534,615 (see particularly col. 19, line 45--col. 22, line 33); and 5,364,769 (see particularly col. 4, line 55--col. 7, line 26), each herein incorporated by reference.

Pharmaceutical compositions containing a compound of the present invention or its pharmaceutically acceptable salts as the active ingredient can be prepared according to conventional pharmaceutical compounding techniques. See, for example, *Remington's Pharmaceutical Sciences*, 18th Ed. (1990, Mack Publishing Co., Easton, PA). Typically, an antagonistic amount of the active ingredient will be admixed with a pharmaceutically acceptable carrier. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous, oral or parenteral. The compositions may further contain antioxidizing agents, stabilizing agents, preservatives and the like. For examples of delivery methods see U.S. Patent No. 5,844,077, incorporated herein by reference.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent can be encapsulated to make it stable to passage through the gastrointestinal tract while at the same time allowing for passage across the blood brain barrier. See for example, WO 96/11698.

For parenteral administration, the compound may be dissolved in a pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intrathecally, they may also be dissolved in cerebrospinal fluid.

The active agent is preferably administered in an therapeutically effective amount. The actual amount administered, and the rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of treatment, e.g. decisions on dosage, timing, etc., is within the responsibility of general practitioners or spealists, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in *Remington's Parmaceutical Sciences*. Typically the active agents of the present invention exhibit their effect at a dosage range from about 0.001 mg/kg to about 250 mg/kg, preferably from about 0.01 mg/kg to about 100 mg/kg of the active ingredient, more preferably from a bout 0.05 mg/kg to about 75 mg/kg. A suitable dose can be administered in multiple sub-doses per day. Typically, a dose or sub-dose may contain from about 0.1 mg to about 500 mg of the active ingredient per unit dosage form. A more preferred dosage will contain

from about 0.5 mg to about 100 mg of active ingredient per unit dosage form. Dosages are generally initiated at lower levels and increased until desired effects are achieved.

Alternatively, targeting therapies may be used to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable for a variety of reasons, e.g. if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

The active agents, which are peptides, can also be administered in a cell based delivery system in which a DNA sequence encoding an active agent is introduced into cells designed for implantation in the body of the patient, especially in the spinal cord region. Suitable delivery systems are described in U.S. Patent No. 5,550,050 and published PCT Application Nos. WO 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635. Suitable DNA sequences can be prepared synthetically for each active agent on the basis of the developed sequences and the known genetic code.

EXAMPLES

The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below were utilized.

EXAMPLE 1

Isolation of τ-Conotoxins

Crude venom was extracted from venom ducts (Cruz et al., 1976), and the components were purified as previously described (Cartier et al., 1996). The crude extract from venom ducts was purified by reverse phase liquid chromatography (RPLC) using a Vydac C_{18} semi-preparative column (10 x 250 mm). Further purification of bioactive peaks was done on a Vydac C_{18} analytical column (4.6 x 220 mm). The effluents were monitored at 220 nm. Peaks were collected, and aliquots were assayed for activity.

The amino acid sequence of the purified peptides were determined by standard methods. The purified peptides were reduced and alkylated prior to sequencing by automated Edman degradation on an Applied Biosystems 477A Protein Sequencer with a 120A Analyzer (DNA/Peptide Facility, University of Utah) (Martinez et al., 1995; Shon et al., 1994).

In accordance with this method, peptides AuVA, AuVB and PVA were obtained.

EXAMPLE 2

Synthesis of Conopeptides

The synthesis of conopeptides, either the mature toxins or the precursor peptides, was separately performed using conventional protection chemistry as described by Cartier et al. (1996). Briefly, the linear chains were built on Rink amide resin by Fmoc procedures with 2-(1H-benzotriol-1-yl)-1,1,3,3,-tetramethyluronium tetrafluoroborated coupling using an ABI model 430A peptide sythesizer with amino acid derivatives purchased from Bachem (Torrence CA). Orthogonal protection was used on cysteines: two cysteines were protected as the stable Cys(S-acetamidomethyl), while the other two cysteines were protected as the acid-labile Cys(S-trityl). After removal of the terminal Fmoc protecting group and cleavage of the peptides from the resins, the released peptides were precipitated by filtering the reaction mixture into -10°C methyl t-butyl ether, which removed the protecting groups except the Cys(S-acetamidomethyl). The peptides were dissolved in 0.1% TFA and 60% acetonitrile and purified by RPLC on a Vydac C_{18} preparative column (22 x 250 mm) and eluted at a flow rate of 20 mL/min with a gradient of acetonitrile in 0.1% TFA.

The disulfide bridges in the three conopeptides were formed as described in Cartier et al. (1996). Briefly, the disulfide bridges between one pair of cysteines were formed by air oxidation which was judged to be complete by analytical RPLC. The monocyclic peptides were purified by RPLC on a Vydac C_{18} prepartive column (22 x 250 mm) and eluted with a gradient of acetonitrile in 0.1% TFA. Removal of S-acetamidomethyl groups and closure of the disulfide bridge between the other pair of cysteines was carried out simultaneously be iodine oxidation. The cyclic peptides were purified by RPLC on a Vydac C_{18} prepartive column (22 x 250 mm) and eluted with a gradient of acetonitrile in 0.1% TFA.

EXAMPLE 3

Isolation of DNA Encoding τ-Conotoxins

DNA coding for τ-conotoxins was isolated and cloned in accordance with conventional techniques using general procedures well known in the art, such as described in Olivera et al. (1996). Alternatively, cDNA libraries was prepared from *Conus* venom duct using conventional techniques. DNA from single clones was amplified by conventional techniques using primers which correspond

approximately to the M13 universal priming site and the M13 reverse universal priming site. Clones having a size of approximately 300-500 nucleotides were sequenced and screened for similarity in sequence to known τ-conotoxins isolated in Example 1. The DNA sequences and encoded propeptide sequences are set forth in Tables 1-15. DNA sequences coding for the mature toxin can also be prepared on the basis of the DNA sequences set forth in these Tables.

TABLE 1

DNA Sequence (SEQ ID NO:20) and Protein Sequence (SEQ ID NO:21) of Tx5.1

```
ggtactcaac gaacttcaag acacattctt ttcacctgga cacgggaagc tgactacaag

caga atg tgc tgt ctc cca gtg ttc gtc att ctt ctg ctg ctg att gca
     Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala

tct gca cct agc gtt gat gcc caa ccg aag acc aaa gat gat gtg ccc
Ser Ala Pro Ser Val Asp Ala Gln Pro Lys Thr Lys Asp Asp Val Pro

ctg gca cct ttg cac gat aat gca aag agt gca cta caa cat ttg aac
Leu Ala Pro Leu His Asp Asn Ala Lys Ser Ala Leu Gln His Leu Asn

caa cgc tgc tgc caa aca ttc tat tgg tgc tgt gtt caa ggg aaa
Gln Arg Cys Cys Gln Thr Phe Tyr Trp Cys Cys Val Gln Gly Lys

tgaatttgga tgagacccct gcgaactgtc catggatgtg agatttggaa agcagactgt

tcctttcgca cgtgttcgtg gaattttgaa tggtcgttaa caacacgctg ccacttgcaa

gctactatct ctctgtcctt tcatctgtgg aactggatga cctaacaact gaaatatcat

agaaattttt cagtgggtat acactatgac catgtagtca gtaattacat catttggacc

ttttgaaata tttttcaaaa tgttaagatt tttccccng gaaaggnctt ttgaagtaaa

tatt
```

TABLE 2

DNA Sequence (SEQ ID NO:22) and Protein Sequence (SEQ ID NO:23) of G5.1

```
atg tgc tgt ctc cca gtc ttc gtc att ctt ctg ttg ctg att aca tct
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Thr Ser

gca cct agc gtt gat gct cta ccg aag acc agg gat gat gtg ccc cta
Ala Pro Ser Val Asp Ala Leu Pro Lys Thr Arg Asp Asp Val Pro Leu

gca tct ttc cac ggt gga tat aat gca agg aga atc cta caa agg cgt
Ala Ser Phe His Gly Gly Tyr Asn Ala Arg Arg Ile Leu Gln Arg Arg

cag ggc tgg tgc tgc aaa gaa aat att gcg tgc tgt ata tagtggtaac
Gln Gly Trp Cys Cys Lys Glu Asn Ile Ala Cys Cys Ile

gggaaatgac tttggatgag acccctgcaa actgtccctg gatgtgaaat ttggaaagta

gactgttcct ttcgcgcgtg ttcgtggaat ttcaaatggt cgtcaacaac acactgctac

ttgcaaagct actatctctc tgtcctttca tctgtggaac tgggtgatct aacagctgaa
```

atgtcgcaga aatttttcaa ttggtctata ctatgaccat gta

TABLE 3

DNA Sequence (SEQ ID NO:24) and Protein Sequence (SEQ ID NO:25) of Qc5.1

```
atg cgc tgt gtc cca gtc ttc atc att ctt ctg ctg ctg agt cca tct
Met Arg Cys Val Pro Val Phe Ile Ile Leu Leu Leu Leu Ser Pro Ser

gca cct agc gtt gat gcc cat ccg atg acc aaa gat gat gtg ccc cag
Ala Pro Ser Val Asp Ala His Pro Met Thr Lys Asp Asp Val Pro Gln

gca tca ttc cat gat gat gca aag cga acc cta caa gta cct tgg atg
Ala Ser Phe His Asp Asp Ala Lys Arg Thr Leu Gln Val Pro Trp Met

aaa cgc ggg tgc tgc gca agg ttg act tgc tgc gtt gga cga
Lys Arg Gly Cys Cys Ala Arg Leu Thr Cys Cys Val Gly Arg

taaagggaaa tgactttgga tgagacccct gcgaactgtc cctggatgtg aaatttggac

agcagactgc tcctttcgca cgtgttcgtg gaattttgaa tggtcgttaa caacacgctg

ccacttgcaa gctattatct ctctgtccct ttatctgtgg aactggataa tctaacaact

gaaatgtcat tgaaaatttt caatggatat atattatgat ccatata
```

TABLE 4

DNA Sequence (SEQ ID NO:26) and Protein Sequence (SEQ ID NO:27) of Im5.1

```
aattcggaag ctgactacaa gcaga atg tac tgt ctc cca gtc ttc atc att
                           Met Tyr Cys Leu Pro Val Phe Ile Ile

ctt ctg ctg ctg att tca tct gca cct agc act cct ccc caa cca agg
Leu Leu Leu Leu Ile Ser Ser Ala Pro Ser Thr Pro Pro Gln Pro Arg

aac aaa gat cgt gtg cac ctg ata tct tta ctc gat aat cac aag caa
Asn Lys Asp Arg Val His Leu Ile Ser Leu Leu Asp Asn His Lys Gln

atc cta caa aga gat tgg aac agt tgc tgt ggg aaa aat cct ggt tgc
Ile Leu Gln Arg Asp Trp Asn Ser Cys Cys Gly Lys Asn Pro Gly Cys

tgt cct tgg gga aaa tgactttgga tgagacccct gcaaactgtc cctggatgtg
Cys Pro Trp Gly Lys

agatttggaa agcagaccgt ttgtggaatt ttgaatggtc gttaacaaca cgctgccact

tgcaagctac aatctctctg tcctttcatc tttggaactg gatgatcaaa caactgaaat

gtcatagaaa tttttcaatg ggtatacaat atgtgggcat ttagtcagta attacatcat

ttgg
```

TABLE 5

DNA Sequence (SEQ ID NO:28) and Protein Sequence (SEQ ID NO:29) of G5.2

```
atg tgc tgt ctc cca gtc ttc gtc att ctt ctg ttg ctg att aca tct
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Thr Ser
```

gca cct agc gtt gat gct cta ccg aag acc agg gat gat gtg ccc cta
Ala Pro Ser Val Asp Ala Leu Pro Lys Thr Arg Asp Asp Val Pro Leu

gca tct ttc cac ggt gga tat aat gca agg aga atc cta caa agg cgt
Ala Ser Phe His Gly Gly Tyr Asn Ala Arg Arg Ile Leu Gln Arg Arg

cag ggc tgg tgc tgc aaa gaa aat att gcg tgc tgt gta tagtggtaac
Gln Gly Trp Cys Cys Lys Glu Asn Ile Ala Cys Cys Val

gggaaatgac tttggatgag acccctgcaa actgtccctg gatgtgaaat ttggaaagta

gactgttcct ttcgcgcgtg ttcgtggaat ttcaaatggt cgtcaacaac acactgctac

ttgcaaagct actatctctc tgtcctttca tctgtggaac tgggtgatct aacagctgaa

atgtcgcaga aatttttcaa ttggtctata ctatgaccat gtagtcag

TABLE 6

DNA Sequence (SEQ ID NO:30) and Protein Sequence (SEQ ID NO:31) of Tx5.2a

atg cgc tgt ttc cca gtc ttc atc att ctt ctg ctg cta att gca tct
Met Arg Cys Phe Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser

gca cct tgc ttt gat gcc cga acg aag acc gat gat gat gtg ccc ctg
Ala Pro Cys Phe Asp Ala Arg Thr Lys Thr Asp Asp Asp Val Pro Leu

tca tct ctc cgc gat aat cta aag cga acg ata cga aca cgc ctg aac
Ser Ser Leu Arg Asp Asn Leu Lys Arg Thr Ile Arg Thr Arg Leu Asn

ata cgc gag tgc tgc gag gat gga tgg tgc tgt act gct gca ccc tta
Ile Arg Glu Cys Cys Glu Asp Gly Trp Cys Cys Thr Ala Ala Pro Leu

aca ggt cgt tagggataaa ggaaaatggc tttggatgag acccctgcga
Thr Gly Arg

attgtccctg gatgtgagat ttggaaagca gactgttcct ttcgcacgtg ttcgtggaat

ttcgaatggt cgttaacaac acgctgccac ttgcaagcca ccatctctct gtcctttcgt

atgtggaact gtatgatcta acaactgaaa tgtcagaaag ttttcagtgg gtatacacta

tgatcgtata

TABLE 7

DNA Sequence (SEQ ID NO:32) and Protein Sequence (SEQ ID NO:33) of Tx5.2b

atg cgc tgt ttc cca gtc ttc atc att ctt ctg ttg cta att gca tct
Met Arg Cys Phe Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser

gca cct tgc ttt gat gcc cga acg aag acc gat gat gat gtg ccc ctg
Ala Pro Cys Phe Asp Ala Arg Thr Lys Thr Asp Asp Asp Val Pro Leu

tca tct ctc cgc gat aat cta aag cga acg ata cga aca cgc ctg aac
Ser Ser Leu Arg Asp Asn Leu Lys Arg Thr Ile Arg Thr Arg Leu Asn

ata cgc ggg tgc tgc gag gat gga tgg tgc tgt act gct gca ccc tta
Ile Arg Gly Cys Cys Glu Asp Gly Trp Cys Cys Thr Ala Ala Pro Leu

aca ggt cgt tagggataaa ggaaaatggc tttggatgag acccctgcaa
Thr Gly Arg

attgtccctg gatgtgagat ttggaaagca gactgttcct ttcgcacgtg ttcgtggaat

ttcgaatggt cgttaacaac acgctgccac ttgcaagcca ccatctctct gtcctttcgt

atgtggaact gtatgatcta acaactgaaa tgtcagaaag ttttcagtgg gtatacacta

tgatcgtata gtcagtaatt

TABLE 8

DNA Sequence (SEQ ID NO:34) and Protein Sequence (SEQ ID NO:35) of Mr5.1

atg cgc tgc ctc cca gtc ttc gtc att ctt ctg ctg ctg att gca tct
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser

gca cct agc gtt gat gcc cga ccg aag acc aaa gat gat atg ccc ctg
Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro Leu

gca tct ttc cat gat aat gca aag cga atc ctg caa ata ctt cag gac
Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln Asp

aga aat ggt tgc tgc aga gca gga gac tgc tgt tca cga ttt gag ata
Arg Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser Arg Phe Glu Ile

aag gaa aat gac ttt gga tgagaccccc gcaaactgtc cttggatgtg
Lys Glu Asn Asp Phe Gly

agatttggaa agcagactgt tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa

caacacgctg ccacttgcaa gctactatct ctctgtcctt ttgtctgtgg aactgtatga

tcaaacaact gaaatgtcat agaaattttt cagtgggtaa acactatgac catgta

TABLE 9

DNA Sequence (SEQ ID NO:36) and Protein Sequence (SEQ ID NO:37) of Mr5.2

ga atg cgc tgc ctc cca gtc ttc gtc att ctt ctg ctg ctg att gca
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala

tct gca cct agc gtt gat gcc cga ccg aag acc aaa gat gat atg ccc
Ser Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro

ctg gca tct ttc cac gat aat gca aag cga atc ctg caa ata ctt cag
Leu Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln

gac aga aat gct tgc tgc ata gta agg cag tgc tgt tgatgatttg
Asp Arg Asn Ala Cys Cys Ile Val Arg Gln Cys Cys

agataaagga aaatgacttt ggatgagacc cctgcaaact gtccctggat gtgagatttg

gaaagcagac tgttcctttc gcacgtgttc gtggaatttc gaatggtcgt taacaacacg

ctgccacttg caagctacta tctctctgtc ctttcatctg tggaactgta tgatcaaaca

actgaaatgt catagaaatt tttcagtggg taaacactat gatcatgtag tcagtaatta

catcatttgg aattccatca agcttatcga taccgtcgac ctcgaggggg ggcccggt

TABLE 10

DNA Sequence (SEQ ID NO:38) and Protein Sequence (SEQ ID NO:39) of Mr5.3

```
atg cgc tgc ctc cca gtc ttt gtc att ctt ctg ctg ctg att gca tct
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser

gca cct agc gtt gat gcc cga ccg aag acc aaa gat gat atg ccc ctg
Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro Leu

gca tct ttc cat gat aat gca aag cga atc ctg caa ata ctt cag gac
Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln Asp

aga aat ggt tgc tgc aga gca gga gac tgc tgt tca tgatttgaga
Arg Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser

taaagggaaa tgactttgga tgagacccct gcaaactgtc cttggatgtg agatttggaa

agcagactgt tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa caacacgctg

ccacttgcaa gctactatct ctctgtcctt tcatctgtgg aactgtatga tcaaacaact
```

TABLE 11

DNA Sequence (SEQ ID NO:40) and Protein Sequence (SEQ ID NO:41) of Ca5.1

```
atg cgc tgt ctc ccg gtc ttc atc att ctt ctg ctg ctg att gca tct
Met Arg Cys Leu Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser

gca cct ggc gtt gat gcc caa ccg aag acc aaa tat aat gcg ccc ctg
Ala Pro Gly Val Asp Ala Gln Pro Lys Thr Lys Tyr Asn Ala Pro Leu

aca tct ctc cac gat aat gca aag ggt ata cta caa gaa cat tgg aac
Thr Ser Leu His Asp Asn Ala Lys Gly Ile Leu Gln Glu His Trp Asn

aaa cgc tgc tgc ccc aga agg ctt gcc tgc tgt att ata gga cgg aaa
Lys Arg Cys Cys Pro Arg Arg Leu Ala Cys Cys Ile Ile Gly Arg Lys

tgaatgattt tgggtgagat ccctgcaaac tgtccctgga tttgaatttt ggaaagcaga

ctgttccttt cgcacgtgtt cgtggaattt cgaatggtcg ttaacaacac gctgccactt

gcaagctact atctctctgt ccttttttctc tgtgaaactg gatggtctaa caactgaaat

gtcatagaaa attttcaatg ggtatactct atgaccatct a
```

TABLE 12

DNA Sequence (SEQ ID NO:42) and Protein Sequence (SEQ ID NO:43) of Ca5.2

```
atg cgc tgt ctc cca gtc ttc atc att ctt ctg ctg ctg att gca tct
Met Arg Cys Leu Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser

gca cct ggc gtt gat gcc caa ccg aag acc aaa tat gat gcg ccc ctg
Ala Pro Gly Val Asp Ala Gln Pro Lys Thr Lys Tyr Asp Ala Pro Leu

aca tct ctc cac gat aat gca aag ggt ata cta caa gaa cat tgg aac
Thr Ser Leu His Asp Asn Ala Lys Gly Ile Leu Gln Glu His Trp Asn

aaa cgc tgc tgc ccc aac aag cct tgc tgt ttt ata gga agg aaa
Lys Arg Cys Cys Pro Asn Lys Pro Cys Cys Phe Ile Gly Arg Lys
```

tgaatgattt tgggtgagac ccctgcaaac tgtccctgga tttgaatttt ggaaagcaga

ctgttccttt cgcacgtgtt cgtggaattt cgaatggtcg ttaacaacac gctgccactt

gcaagctact atctctctgt ccttttttctc tgtgaaactg gatggtctaa caactgagat

gtcatagaaa attttcaatc ggtgtactct atgaccatct a

TABLE 13

DNA Sequence (SEQ ID NO:44) and Protein Sequence (SEQ ID NO:45) of Qc5.2

atg cgc tgt gtc cca gtc ttc atc att ctt ctg ctg ctg agt cca tct
Met Arg Cys Val Pro Val Phe Ile Ile Leu Leu Leu Leu Ser Pro Ser

gca cct agc gtt gat gcc cat ccg atg acc aaa gat gat gta ccc cag
Ala Pro Ser Val Asp Ala His Pro Met Thr Lys Asp Asp Val Pro Gln

gca tct ctc cat gat gat gca aag cga acc cta caa gta cct tgg atg
Ala Ser Leu His Asp Asp Ala Lys Arg Thr Leu Gln Val Pro Trp Met

aaa cgc ggg tgc tgc gca atg ttg act tgc tgc gtt gga cga
Lys Arg Gly Cys Cys Ala Met Leu Thr Cys Cys Val Gly Arg

taaagggaaa tgactttgga tgagacccct acgaactgtc cctggatgtg aaatttggac

agcagactgc tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa caacacgctg

ccacttgcaa gctattatct ctctgtccct ttatctgtgg aactggataa tctaacaact

gaaacgtcat tgaaaatttt caatggatat atattatgat ccatata

TABLE 14

DNA Sequence (SEQ ID NO:46) and Protein Sequence (SEQ ID NO:47) of Gm5.1

gggcaggtac tcaacgaact tcaggacaca ttcttttcac ctggacacgg gaaactgact

ataagcaga atg cgc tac cta cca gtc ttc gtc att ctt ctg ctg ctg att
Met Arg Tyr Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile

gca tct ata cct agc gat act gtc caa ctg aag acc aaa gat gat atg
Ala Ser Ile Pro Ser Asp Thr Val Gln Leu Lys Thr Lys Asp Asp Met

ccc ctg gca tct ttc cac ggt aat gga aga cga atc ctg cga atg ctt
Pro Leu Ala Ser Phe His Gly Asn Gly Arg Arg Ile Leu Arg Met Leu

tca aac aaa cgc tta tgc tgt gtc acc gag gat tgg tgc tgt gaa tgg
Ser Asn Lys Arg Leu Cys Cys Val Thr Glu Asp Trp Cys Cys Glu Trp

tgg taaaggaaaa tgactttgga tgagacccct gcaaactgtt tctggatgtg
Trp

agatttggaa agcagactgt tctttcgcac gtattcgtga aatttcgaat ggtcgttaac

aacacgctgc cacttgcaag ctgctatctc tctgtctttt catctgtgga actgtatgat

ctaacaactg aaatgtcata gacatttttc attgggtata cactatgacc atgtagccag

taattacatc atttggacct tttggatatt tttcagtatg taagtgtgtt cccttaaaaa

gtcctttgta attatgtatt ttaanaattt angttttgca cataaattgt aaaacgctgt

cctttctgtt gntcctacat cantggtggg gaaaagnaaa àtgtttggcc ntggtcaaat

ttaaataatn accctgccgt ttnaatgcng ttattantgg tattttnaac nttgnacggt

taaactt

TABLE 15

DNA Sequence (SEQ ID NO:48) and Protein Sequence (SEQ ID NO:49) of Gm5.2

```
ga atg cgc tgt ctc cca gtc ttc gtc att ctt ctg ctg ctg att gca
   Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala

tct gca cct agc gtt gat gcc caa ccg aag acc aaa gat gat gtg ccc
Ser Ala Pro Ser Val Asp Ala Gln Pro Lys Thr Lys Asp Asp Val Pro

ctg gca cct ttg cac gat aat ata agg agt act cta caa aca ctt cgg
Leu Ala Pro Leu His Asp Asn Ile Arg Ser Thr Leu Gln Thr Leu Arg

aag aaa gtc tgc tgc cgc cca gtg cag gat tgc tgt tca ggg aaa
Lys Lys Val Cys Cys Arg Pro Val Gln Asp Cys Cys Ser Gly Lys

tgaagggaaa tgaatttgga tgagacccct gcgaactgtc cctggatgtg agatttggaa

agcagactgt tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa caacacgctg

ccacttgcaa gctactatct ctctgtcctt tcatctgcgg aactggatga cctaaagctt

gtgatc
```

EXAMPLE 4

Biological Activity of τ-Conotoxins

The biological activity of τ-conotoxin peptides at the acetylcholine receptor was tested in the fluorescence assay as described by Cornell-Bell et al. (1999). Briefly, primary cortical cells are exposed to acetylcholine in the presence or absence of a τ-conotoxin peptide. Acetylcholine causes the primary cortical cells to flux calcium, which is measured by increases in fluorescence in cells loaded with Fluo-3, a calcium imaging dye. The τ-conotoxin peptide AuVA inhibited the response of primary cortical cells to acetylcholine at low concentrations (10 pM) at 15 seconds following exposure to the peptide and acetylcholine. This study shows that the τ-conotoxin peptide act at the acetylcholine receptor.

EXAMPLE 5

Effect of τ-Conotoxins in a Pain Model

The effect of τ-conotoxin peptides for use in treating pain was by testing in two pain models, the first being the hind-paw licking model (Woolfe and MacDonald, 1944; Plummer et al., 1991; Suh et al., 1992; Plone et al., 1996) and the second being the accelerating roto-rod model. In the

hind-paw licking model, it was found that 10 nmol of τ-conotoxin peptide AuVA increased the latency to initiate hind-paw licking in mice on a 55° hot plate 15 minutes following freehand i.c.v. injection. It was further found that 1nmol τ-conotoxin peptide AuVA did not have any effect in this model. In the accelerating roto-rod model, it was found that τ-conotoxin peptide AuVA produced impairment of motor performance following injection of τ-conotoxin peptide AuVA. The effects seen in these models demonstrates that the τ-conotoxin peptides have analgesic properties.

It will be appreciated that the methods and compositions of the instant invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

LIST OF REFERENCES

Barnay, G. et al. (2000). *J. Med. Chem.*

Bitan, G. et al. (1997). *J. Peptide Res.* **49**:421-426.

Bodansky et al. (1966). *Chem. Ind.* **38**:1597-98.

Cartier, G.E. et al. (1996). *J. Biol. Chem.* **271**:7522-7528.

Cornell-Bell, A.H. et al. (1999). Kainate spiral waves and integrins: A signaling system without gap junctions. *Glia*, in press.

Cruz, L.J. at al. (1976). *Verliger* **18**:302-308.

Cruz, L.J. et al. (1987). *J. Biol. Chem.* **260**:9280-9288.

Haack, J.A. et al. (1990). *J. Biol. Chem.* **265**:6025-6029.

Hammerland et al. (1992). *Eur. J. Pharmacol.* **226**:239-244.

Horiki, K. et al. (1978). *Chemistry Letters* 165-68.

Hubry, V. et al. (1994). *Reactive Polymers* **22**:231-241.

Kapoor (1970). *J. Pharm. Sci.* **59**:1-27.

Kornreich, W.D. et al. (1986). U.S. Patent No. 4,569,967.

Martinez, J.S. et al. (1995). *Biochem.* **34**:14519-14526.

McIntosh, J.M. et al. (1982). *Arch. Biochem. Biophys.* **218**:329-334.

Mena, E.E. et al. (1990). *Neurosci. Lett.* **118**:241-244.

Methoden der Organischen Chemie (Houben-Weyl): Synthese von Peptiden, E. Wunsch (Ed.), Georg Thieme Verlag, Stuttgart, Ger. (1974).

Myers, R.A. et al. (1991). *Biochemistry* **30**:9370-9377.

Nishiuchi, Y. et al. (1993). *Int. J. Pept. Protein Res.* **42**:533-538.

Nowak, L. et al. (1984). *Nature* **307**:462-465.

Olivera, B.M. et al. (1984). U.S. Patent 4,447,356.

Olivera, B.M. et al. (1985). *Science* **230**:1338-1343.

Olivera, B.M. et al. (1996). U.S. Patent 5,514,774.

Ornstein, et al. (1993). *Biorganic Medicinal Chemistry Letters* **3**:43-48.

Plone, M. A. et al. (1996). *Pain* **66**:265-70.

Plummer, J. L. et al. (1991). *J Pharmacol Methods* **26**:79-87.

Rivier, J.R. et al. (1978). *Biopolymers* **17**:1927-38.

Rivier, J.R. et al. (1987). *Biochem.* **26**:8508-8512.

Sambrook, J. et al. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Schroder & Lubke (1965). *The Peptides* **1**:72-75, Academic Press, NY.

Shon, K.-J. et al. (1994). *Biochemistry* **33**:11420-11425.

Stewart and Young, *Solid-Phase Peptide Synthesis*, Freeman & Co., San Francisco, CA (1969).

Suh, H.H. et al. (1992). *Eur J Pharmacol* **213**:337-41.

Vale et al. (1978). U.S. Patent 4,105,603.

Van de Steen, P. et al. (1998). *Critical Rev. in Biochem. and Mol. Biol.* **33**:151-208.

Woolfe, G. and MacDonald, A. (1944). *J. Pharmacol. Exp. Ther.* **80**:300-307.

Zafaralla, G.C. et al. (1988). *Biochemistry* **27**:7102-7105.

Zhou L.M., et al. (1996). *J. Neurochem.* **66**:620-628.

U.S. Patent No. 3,972,859.

U.S. Patent No. 3,842,067.

U.S. Patent No. 3,862,925.

U.S. Patent No. 5,514,774.

U.S. Patent No. 5,531,001.

U.S. Patent No. 5,534,615.

U.S. Patent No. 5,364,769.

U.S. Patent No. 5,545,723.

U.S. Patent No. 5,550,050.

U.S. Patent No. 5,591,821.

U.S. Patent No. 5,719,264.

U.S. Patent No. 5,844,077.

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PCT Published Application WO 94/25503.

PCT Published Application WO 95/01203.

PCT Published Application WO 95/05452.

PCT Published Application WO 96/02286.

PCT Published Application WO 96/02646.

PCT Published Application WO 96/11698.

PCT Published Application WO 96/40871.

PCT Published Application WO 96/40959.

PCT Published Application WO 97/12635.

PCT Published Application WO 98/03189.

WHAT IS CLAIMED IS:

1. A substantially pure τ-conotoxin peptide having the generic formula I: Xaa_1-Xaa_2-Xaa_3-Xaa_4-Cys-Cys-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Cys-Cys-Xaa_{10}-Xaa_{11}-Xaa_{12}-Xaa_{13}-Xaa_{14}-Xaa_{15}-Xaa_{16}-Xaa_{17}-Xaa_{18}-Xaa_{19} (SEQ ID NO:1), wherein Xaa_1 is des-Xaa_1, Asp, Glu or γ-carboxy-Glu (Gla); Xaa_2 is des-Xaa_2, Gln, Asn, Glu, Trp (D or L), neo-Trp, halo-Trp or any unnatural aromatic amino acid; Xaa_3 is des-Xaa_3, Gly, Ala, Asn or Gln; Xaa_4 is des-Xaa_4,Val, Leu (D or L), Ile, Ala, Gly, Glu, Gla, Asp, Ser, Thr, Phe, Trp (D or L), neo-Trp, halo-Trp (D or L) or any unnatural aromatic amino acid; Xaa_5 is Pro, hydroxy-Pro, Gln, Asn, Glu, Gla, Ala, Gly, Lys, Arg, Ile, Val, homoarginine, ornithine, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; Xaa_6 is Val, Phe, Thr, Ser, Glu, Gla, Asp, Asn, Gln, Ala, Gly, Ile, Leu (D or L), Met, Pro, hydroxy-Pro, Arg, homoarginine, ornithine, Lys, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa_7 is any Val, Ile, Asn, Leu (D or L), Gln, Gly, Ala, Phe, Glu, Gla, Arg, ornithine, homoarginine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa_8 is Ile, Leu (D or L), Met, Thr, Ser, Pro, hydroxy-Pro, Gln, Asp, Glu, Gla, Asn, Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr, nitro-Tyr, any unnatural basic amino acid, any unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa_9 is des-Xaa_9, Ala, Gly, Asp, Glu, Gla, Trp (D or L) neo-Trp, halo-Trp (D or L), Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Arg, homoarginine, ornithine, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr, nitro-Tyr or any unnatural basic amino acid; Xaa_{10} is des-Xaa_{10}, Ile, Leu (D or L), Val, Glu, Gla, Asp, Thr, Ser, Pro, hydroxy-Pro, Trp (D or L), neo-Trp, halo-Trp (D or L), Phe, any unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa_{11} is des-Xaa_{11}, Gln, Asn, Leu (D or L), Ile, Val, Ala, Gly, Trp (D or L), neo-Trp, halo-Trp (D or L), Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa_{12} is des-Xaa_{12}, Ala, Gly, Phe, Trp (D or L), neo-Trp, halo-Trp (D or L) or any unnatural aromatic amino acid; Xaa_{13} is des-Xaa_{13}, Glu, Gla, Asp, Phe or any unnatural aromatic amino acid; Xaa_{14} is des-Xaa_{14}, Ile, Val or Leu (D or L); Xaa_{15} is des-Xaa_{15}, Thr, Ser, Arg,

homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; Xaa_{16} is des-Xaa_{16}, Glu, Gla or Asp; Xaa_{17} is des-Xaa_{17}, Asn or Gln; Xaa_{18} is des-Xaa_{18}, Asp, Glu or Gla; Xaa_{19} is des-Xaa_{19}, Phe or any unnatural aromatic amino acid; and the C-terminus contains a free carboxyl group or an amide group.

2. A substantially pure τ-conotoxin peptide seleceted from the group consisting of:

Phe-Cys-Cys-Xaa_1-Val-Ile-Arg-Xaa_2-Cys-Cys-Xaa_3 (SEQ ID NO:2);

Phe-Cys-Cys-Xaa_1-Phe-Ile-Arg-Xaa_2-Cys-Cys-Xaa_3 (SEQ ID NO:3);

Cys-Cys-Gln-Thr-Phe-Xaa_2-Xaa_3-Cys-Cys-Gln (SEQ ID NO:4);

Xaa_4-Gly-Xaa_3-Cys-Cys-Xaa_5-Xaa_6-Asn-Ile-Ala-Cys-Cys-Ile (SEQ ID NO:5);

Gly-Cys-Cys-Ala-Arg-Leu-Thr-Cys-Cys-Val (SEQ ID NO:6);

Asn-Gly-Cys-Cys-Xaa_1-Xaa_5-Gln-Met-Arg-Cys-Cys-Thr (SEQ ID NO:7);

Asp-Xaa_3-Asn-Ser-Cys-Cys-Gly-Xaa_5-Asn-Xaa_1-Gly-Cys-Cys-Xaa_1-Xaa_3 (SEQ ID NO:8);

Xaa_4-Gly-Xaa_3-Cys-Cys-Xaa_5-Xaa_6-Asn-Ile-Arg-Cys-Cys-Val (SEQ ID NO:9);

Xaa_6-Cys-Cys-Xaa_6-Asp-Gly-Xaa_3-Cys-Cys-Thr-Ala-Ala-Xaa_1-Leu-Thr (SEQ ID NO:10);

Gly-Cys-Cys-Xaa_6-Asp-Gly-Xaa_3-Cys-Cys-Thr-Ala-Ala-Xaa_1-Leu-Thr (SEQ ID NO:11);

Asn-Gly-Cys-Cys-Arg-Ala-Gly-Asp-Cys-Cys-Ser-Arg-Phe-Xaa_6-Ile-Xaa_5-Xaa_6-Asn-Asp-Phe (SEQ ID NO:12);

Asn-Ala-Cys-Cys-Ile-Val-Arg-Gln-Cys-Cys (SEQ ID NO:13);

Asn-Gly-Cys-Cys-Arg-Ala-Gly-Asp-Cys-Cys-Ser (SEQ ID NO:14);

Cys-Cys-Xaa_1-Arg-Arg-Leu-Ala-Cys-Cys-Ile-Ile (SEQ ID NO:15);

Cys-Cys-Xaa_1-Asn-Xaa_5-Xaa_1-Cys-Cys-Phe-Ile (SEQ ID NO:16);

Gly-Cys-Cys-Ala-Met-Leu-Thr-Cys-Cys-Val (SEQ ID NO:17);

Leu-Cys-Cys-Val-Thr-Xaa_6-Asp-Xaa_3-Cys-Cys-Xaa_6-Xaa_3-Xaa_3 (SEQ ID NO:18); and

Val-Cys-Cys-Arg-Xaa_1-Val-Gln-Asp-Cys-Cys-Ser (SEQ ID NO:19);

wherein Xaa_1 is Pro or hydroxy-Pro; Xaa_2 is Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr or nitro-Tyr; Xaa_3 is Trp or halo-Trp; Xaa_4 is Gln or pyro-Glu; Xaa_5 is

Lys, N-methyl-Lys, N,N-dimethyl-Lys or N,n,N-trimethyl-Lys, Xaa_6 is Glu or gamma-carboxy-Glu (Gla); and the C-terminus contains a carboxyl or amide group.

3. The substantially pure τ-conotoxin peptide of claim 2, wherein Xaa_6 is Glu.

4. The substantially pure τ-conotoxin peptide of claim 2, wherein Xaa_5 is Lys.

5. The substantially pure τ-conotoxin peptide of claim 2, wherein Xaa_4 is Gln.

6. The substantially pure τ-conotoxin peptide of claim 2, wherein Xaa_2 is Tyr.

7. The substantially pure τ-conotoxin peptide of claim 2, wherein Xaa_2 is mono-iodo-Tyr.

8. The substantially pure τ-conotoxin peptide of claim 2, wherein Xaa_2 is di-iodo-Tyr.

9. The substantially pure τ-conotoxin peptide of claim 2, wherein Xaa_3 is Trp.

10. The substantially pure α-conotoxin peptide of claim 2, wherein Xaa_1 is Pro or hydroxy-Pro, Xaa_2 is Tyr, mono-iodo-Tyr or di-iodo-Tyr, Xaa_3 is Trp, Xaa_4 is Gln, Xaa_5 is Lys and Xaa_6 is Glu.

11. The substantially pure α-conotoxin peptide of claim 1, which is modified to contain an O-glycan, an S-glycan or an N-glycan.

12. The substantially pure α-conotoxin peptide of claim 2 which is modified to contain an O-glycan, an S-glycan or an N-glycan.

13. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:2, wherein Xaa_1 is Pro, Xaa_2 is Tyr and Xaa_3 is Trp.

14. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:3, wherein Xaa_1 is Pro, Xaa_2 is Tyr and Xaa_3 is Trp.

15. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:4, wherein Xaa_2 is Tyr and Xaa_3 is Trp.

16. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:5, wherein wherein Xaa_3 is Trp, Xaa_4 is Gln, Xaa_5 is Lys and Xaa_6 is Glu.

17. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:6.

18. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:7, wherein Xaa_1 is Pro and Xaa_5 is Lys.

19. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:8, wherein Xaa_1 is Pro, Xaa_3 is Trp and Xaa_5 is Lys.

20. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:9, wherein Xaa_3 is Trp, Xaa_4 is Gln, Xaa_5 is Lys and Xaa_6 is Glu.

21. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:10, wherein Xaa_1 is Pro, Xaa_3 is Trp and Xaa_6 is Glu.

22. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:11, wherein Xaa_1 is Pro, Xaa_3 is Trp and Xaa_6 is Glu.

23. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:12, wherein Xaa_5 is Lys and Xaa_6 is Glu.

24. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:13.

25. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:14.

26. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:15, wherein Xaa_1 is Pro.

27. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:16, wherein Xaa_1 is Pro and Xaa_5 is Lys.

28. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:17.

29. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:18, wherein Xaa_3 is Trp and Xaa_6 is Glu.

30. The substantially pure τ-conotoxin peptide of claim 2, wherein said peptide has the sequence set forth in SEQ ID NO:19, wherein Xaa_1 is Pro.

31. An isolated nucleic acid comprising a nucleic acid coding for a τ-conotoxin precursor comprising an amino acid sequence selected from the group of amino acid sequences set forth in Tables 1-15.

32. The nucleic acid of claim 29 wherein the nucleic acid comprises a nucleotide sequence selected from the group of nucleotide sequences set forth in Tables 1-15 or their complements.

33. A substantially pure τ-conotoxin protein precursor comprising an amino acid sequence selected from the group of amino acid sequences set forth in Tables 1-15.

34. A pharmaceutical composition comprising a τ-conotoxin peptide or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier, said τ-conotoxin peptide having the generic formula I: Xaa_1-Xaa_2-Xaa_3-Xaa_4-Cys-Cys-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-

Cys-Cys-Xaa$_{10}$-Xaa$_{11}$-Xaa$_{12}$-Xaa$_{13}$-Xaa$_{14}$-Xaa$_{15}$-Xaa$_{16}$-Xaa$_{17}$-Xaa$_{18}$-Xaa$_{19}$ (SEQ ID NO:1), wherein Xaa$_1$ is des-Xaa$_1$, Asp, Glu or γ-carboxy-Glu (Gla); Xaa$_2$ is des-Xaa$_2$, Gln, Asn, Glu, Trp (D or L), neo-Trp, halo-Trp or any unnatural aromatic amino acid; Xaa$_3$ is des-Xaa$_3$, Gly, Ala, Asn or Gln; Xaa$_4$ is des-Xaa$_4$,Val, Leu (D or L), Ile, Ala, Gly, Glu, Gla, Asp, Ser, Thr, Phe, Trp (D or L), neo-Trp, halo-Trp (D or L) or any unnatural aromatic amino acid; Xaa$_5$ is Pro, hydroxy-Pro, Gln, Asn, Glu, Gla, Ala, Gly, Lys, Arg, Ile, Val, homoarginine, ornithine, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; Xaa$_6$ is Val, Phe, Thr, Ser, Glu, Gla, Asp, Asn, Gln, Ala, Gly, Ile, Leu (D or L), Met, Pro, hydroxy-Pro, Arg, homoarginine, ornithine, Lys, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa$_7$ is any Val, Ile, Asn, Leu (D or L), Gln, Gly, Ala, Phe, Glu, Gla, Arg, ornithine, homoarginine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa$_8$ is Ile, Leu (D or L), Met, Thr, Ser, Pro, hydroxy-Pro, Gln, Asp, Glu, Gla, Asn, Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr, nitro-Tyr, any unnatural basic amino acid, any unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa$_9$ is des-Xaa$_9$, Ala, Gly, Asp, Glu, Gla, Trp (D or L) neo-Trp, halo-Trp (D or L), Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Arg, homoarginine, ornithine, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr, nitro-Tyr or any unnatural basic amino acid; Xaa$_{10}$ is des-Xaa$_{10}$, Ile, Leu (D or L), Val, Glu, Gla, Asp, Thr, Ser, Pro, hydroxy-Pro, Trp (D or L), neo-Trp, halo-Trp (D or L), Phe, any unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa$_{11}$ is des-Xaa$_{11}$, Gln, Asn, Leu (D or L), Ile, Val, Ala, Gly, Trp (D or L), neo-Trp, halo-Trp (D or L), Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa$_{12}$ is des-Xaa$_{12}$, Ala, Gly, Phe, Trp (D or L), neo-Trp, halo-Trp (D or L) or any unnatural aromatic amino acid; Xaa$_{13}$ is des-Xaa$_{13}$, Glu, Gla, Asp, Phe or any unnatural aromatic amino acid; Xaa$_{14}$ is des-Xaa$_{14}$, Ile, Val or Leu (D or L); Xaa$_{15}$ is des-Xaa$_{15}$, Thr, Ser, Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural basic amino acid; Xaa$_{16}$ is des-Xaa$_{16}$, Glu, Gla or Asp; Xaa$_{17}$ is des-Xaa$_{17}$, Asn or Gln; Xaa$_{18}$ is des-Xaa$_{18}$,

Asp, Glu or Gla; Xaa_{19} is des-Xaa_{19}, Phe or any unnatural aromatic amino acid; and the C-terminus contains a free carboxyl group or an amide group.

35. The pharmaceutical composition of claim 34, which is modified to contain an O-glycan, an S-glycan or an N-glycan.

36. A pharmaceutical composition comprising a τ-conotoxin peptide or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier, said τ-conotoxin peptide seleceted from the group consisting of:

Phe-Cys-Cys-Xaa_1-Val-Ile-Arg-Xaa_2-Cys-Cys-Xaa_3 (SEQ ID NO:2);

Phe-Cys-Cys-Xaa_1-Phe-Ile-Arg-Xaa_2-Cys-Cys-Xaa_3 (SEQ ID NO:3);

Cys-Cys-Gln-Thr-Phe-Xaa_2-Xaa_3-Cys-Cys-Gln (SEQ ID NO:4);

Xaa_4-Gly-Xaa_3-Cys-Cys-Xaa_5-Xaa_6-Asn-Ile-Ala-Cys-Cys-Ile (SEQ ID NO:5);

Gly-Cys-Cys-Ala-Arg-Leu-Thr-Cys-Cys-Val (SEQ ID NO:6);

Asn-Gly-Cys-Cys-Xaa_1-Xaa_5-Gln-Met-Arg-Cys-Cys-Thr (SEQ ID NO:7);

Asp-Xaa_3-Asn-Ser-Cys-Cys-Gly-Xaa_5-Asn-Xaa_1-Gly-Cys-Cys-Xaa_1-Xaa_3 (SEQ ID NO:8);

Xaa_4-Gly-Xaa_3-Cys-Cys-Xaa_5-Xaa_6-Asn-Ile-Arg-Cys-Cys-Val (SEQ ID NO:9);

Xaa_6-Cys-Cys-Xaa_6-Asp-Gly-Xaa_3-Cys-Cys-Thr-Ala-Ala-Xaa_1-Leu-Thr (SEQ ID NO:10);

Gly-Cys-Cys-Xaa_6-Asp-Gly-Xaa_3-Cys-Cys-Thr-Ala-Ala-Xaa_1-Leu-Thr (SEQ ID NO:11);

Asn-Gly-Cys-Cys-Arg-Ala-Gly-Asp-Cys-Cys-Ser-Arg-Phe-Xaa_6-Ile-Xaa_5-Xaa_6-Asn-Asp-Phe (SEQ ID NO:12);

Asn-Ala-Cys-Cys-Ile-Val-Arg-Gln-Cys-Cys (SEQ ID NO:13);

Asn-Gly-Cys-Cys-Arg-Ala-Gly-Asp-Cys-Cys-Ser (SEQ ID NO:14);

Cys-Cys-Xaa_1-Arg-Arg-Leu-Ala-Cys-Cys-Ile-Ile (SEQ ID NO:15);

Cys-Cys-Xaa_1-Asn-Xaa_5-Xaa_1-Cys-Cys-Phe-Ile (SEQ ID NO:16);

Gly-Cys-Cys-Ala-Met-Leu-Thr-Cys-Cys-Val (SEQ ID NO:17);

Leu-Cys-Cys-Val-Thr-Xaa_6-Asp-Xaa_3-Cys-Cys-Xaa_6-Xaa_3-Xaa_3 (SEQ ID NO:18); and

Val-Cys-Cys-Arg-Xaa_1-Val-Gln-Asp-Cys-Cys-Ser (SEQ ID NO:19);

wherein Xaa_1 is Pro or hydroxy-Pro; Xaa_2 is Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr or nitro-Tyr; Xaa_3 is Trp or halo-Trp; Xaa_4 is Gln or pyro-Glu; Xaa_5 is Lys, N-methyl-Lys, N,N-dimethyl-Lys or N,n,N-trimethyl-Lys, Xaa_6 is Glu or gamma-carboxy-Glu (Gla); and the C-terminus contains a carboxyl or amide group.

37. The pharmaceutical composition of claim 36 which is modified to contain an O-glycan, an S-glycan or an N-glycan.

SEQUENCE LISTING

<110> University of Utah Research Foundation
Cognetix, Inc.

<120> Tau-Conotoxin Peptides

<130> Tau-Conopeptides

<140>
<141>

<150> US 60/118,642
<151> 1999-02-04

<160> 49

<170> PatentIn Ver. 2.0

<210> 1
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Generic Sequence for Tau Conopeptides

<220>
<221> PEPTIDE
<222> (1)..(2)
<223> Xaa at residue 1 is Asp, Glu or gamma-carboxy-Glu (Gla); Xaa at residue 2 is des-Xaa, Gln, Asn, Glu, Trp (D or L), neo-Trp, halo-Trp, any unnatural aromatic amino acid.

<220>
<221> PEPTIDE
<222> (3)..(4)
<223> Xaa at residue 3 is des-Xaa, Gly, Ala, Asn or Gln; Xaa at residue 4 is des-Xaa4,Val, Leu (D or L), Ile, Ala, Gly, Glu, Gla, Asp, Ser, Thr, Phe, Trp (D or L), neo-Trp, halo-Trp (D or L) or any

<220>
<221> PEPTIDE
<222> (4)..(7)
<223> unnatural aromatic amino acid; Xaa at residue 7 is Pro, hydroxy-Pro, Gln, Asn, Glu, Gla, Ala, Gly, Lys, Arg, Ile, Val, homoarginine, ornithine, N-methyl-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or

<220>
<221> PEPTIDE
<222> (7)..(8)
<223> any unnatural basic amino acid; Xaa at residue 8 is Val, Phe, Thr, Ser, Glu, Gla, Asp, Asn, Gln, Ala, Gly, Ile, Leu (D or L), Met, Pro, hydroxy-Pro, Arg, homoarginine, ornithine, Lys, N-methyl-Lys,

<220>
<221> PEPTIDE

<222> (8)..(9)
<223> N,N,-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa at residue 9 is Val, Ile, Asn, Leu (D or L), Gln, Gly, Ala, Phe, Glu, Gla, Arg,

<220>
<221> PEPTIDE
<222> (9)..(10)
<223> ornithine, arginine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural aromatic amino acid; Xaa at residue 10 is Ile, Leu (D or L), Met, Thr,

<220>
<221> PEPTIDE
<222> (10)
<223> Ser, Pro, hydroxy-Pro, Gln, Asp, Glu, Gla, Asn, Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr,

<220>
<221> PEPTIDE
<222> (10)..(11)
<223> O-phosopho-Tyr, nitro-Tyr, any unnatural basic amino acid, any unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa at residue 11 is des-Xaa, Ala, Gly, Asp, Glu, Gla,

<220>
<221> PEPTIDE
<222> (11)
<223> Trp (D or L), neo-Trp, halo-Trp (D or L), Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, Arg, homoarginine, ornithine, Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr,

<220>
<221> PEPTIDE
<222> (11)..(14)
<223> nitro-Tyr or any unnatural basic amino acid; Xaa at residue 14 is des-Xaa, Ile, Leu (D or L), Val, Glu, Gla, Asp, Thr, Ser, Pro, hydroxy-Pro, Trp (D or L), neo-Trp, halo-Trp (D or L), Phe, any

<220>
<221> PEPTIDE
<222> (14)..(15)
<223> unnatural aromatic amino acid or any unnatural hydroxy containing amino acid; Xaa at residue 15 is des-Xaa11, Gln, Asn, Leu (D or L), Ile, Val, Ala, Gly, Trp (D or L), neo-Trp, halo-Trp (D or L), Arg,

<220>
<221> PEPTIDE
<222> (15)..(16)
<223> homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys, any unnatural basic amino acid or any unnatural

aromatic amino acid; Xaa at residue 16 is des-Xaa, Ala, Gly, Phe,

<220>
<221> PEPTIDE
<222> (16)..(17)
<223> Trp (D or L), neo-Trp, halo-Trp (D or L) or any unnatural aromatic amino acid; Xaa at residue 17 is des-Xaa, Glu, Gla, Asp, Phe or any unnatural aromatic amino acid.

<220>
<221> PEPTIDE
<222> (18)..(19)
<223> Xaa at residue 18 is des-Xaa, Ile, Val or Leu (D or L); Xaa at residue 19 is des-Xaa, Thr, Ser, Arg, homoarginine, ornithine, Lys, N-methy-Lys, N,N-dimethyl-Lys, N,N,N-trimethyl-Lys or any unnatural

<220>
<221> PEPTIDE
<222> (19)..(22)
<223> basic amino acid; Xaa at residue 20 is des-Xaa, Glu, Gla or Asp; Xaa at residue 21 is des-Xaa, Asn or Gln; Xaa at residue 22 is des-Xaa, Asp, Glu or Gla.

<220>
<221> PEPTIDE
<222> (23)
<223> Xaa at residue 23 is des-Xaa, Phe or any unnatural aromatic amino acid.

<400> 1
Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20

<210> 2
<211> 11
<212> PRT
<213> Conus aulicus

<220>
<221> PEPTIDE
<222> (4)..(8)
<223> Xaa at residue 4 is Pro or hydroxy-Pro; Xaa at residue 8 is Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr or nitro-Tyr.

<220>
<221> PEPTIDE
<222> (11)
<223> Xaa at residue 11 is Trp (D or L), neo-Trp or halo-Trp (D or L).

<400> 2
Phe Cys Cys Xaa Val Ile Arg Xaa Cys Cys Xaa
1 5 10

<210> 3
<211> 11
<212> PRT
<213> Conus aulicus

<220>
<221> PEPTIDE
<222> (4)..(8)
<223> Xaa at residue 4 is Pro or hydroxy-Pro; Xaa at residue 8 is Tyr, nor-Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr or nitro-Tyr.

<220>
<221> PEPTIDE
<222> (11)
<223> Xaa at residue 11 is Trp (D or L), neo-Trp or halo-Trp (D or L).

<400> 3
Phe Cys Cys Xaa Phe Ile Arg Xaa Cys Cys Xaa
1 5 10

<210> 4
<211> 10
<212> PRT
<213> Conus textile

<220>
<221> PEPTIDE
<222> (6)..(7)
<223> Xaa at residue 6 is Tyr, mono-halo-Tyr, di-halo-Tyr, O-sulpho-Tyr, O-phospho-Tyr or nitro-Tyr; Xaa at residue 7 is Trp (D or L), neo-Trp or halo-Trp (D or L).

<400> 4
Cys Cys Gln Thr Phe Xaa Xaa Cys Cys Gln
1 5 10

<210> 5
<211> 13
<212> PRT
<213> Conus geographus

<220>
<221> PEPTIDE
<222> (1)..(3)
<223> Xaa at residue 1 is Gln or pyro-Glu; Xaa at residue 3 is Trp (D or L), neo-Trp or halo-Trp (D or L); Xaa at residue 6 is Lys, N-methyl-Lys, N,N-dimethyl-Lys or N,N,N-trimethyl-Lys.

<220>
<221> PEPTIDE
<222> (7)
<223> Xaa at residue 7 is Glu or gamma-carboxy-Glu.

<400> 5
Xaa Gly Xaa Cys Cys Xaa Xaa Asn Ile Ala Cys Cys Ile
1 5 10

<210> 6
<211> 10
<212> PRT
<213> Conus quercinus

<400> 6
Gly Cys Cys Ala Arg Leu Thr Cys Cys Val
1 5 10

<210> 7
<211> 12
<212> PRT
<213> Conus purpurascens

<220>
<221> PEPTIDE
<222> (5)..(6)
<223> Xaa at residue 5 is Pro or hydroxy-Pro; Xaa at residue 6 is Lys, N-methyl-Lys, N,N-dimethyly-Lys or N,N,N-trimethyl-Lys.

<400> 7
Asn Gly Cys Cys Xaa Lys Gln Met Arg Cys Cys Thr
1 5 10

<210> 8
<211> 15
<212> PRT
<213> Conus imperialis

<220>
<221> PEPTIDE
<222> (2)..(15)
<223> Xaa at residues 2 and 15 is Trp (D or L), neo-Trp or halo-Trp (D or L); Xaa at residue 8 is Lys, N-methyl-Lys, N,N-dimethyl-Lys or N,N,N-trimethyl-Lys; Xaa at residues 10 and 14 is Pro or hydroxy-Pro.

<400> 8
Asp Xaa Asn Ser Cys Cys Gly Xaa Asn Xaa Gly Cys Cys Xaa Xaa
1 5 10 15

<210> 9
<211> 13
<212> PRT
<213> Conus geographus

<220>
<221> PEPTIDE
<222> (1)..(6)
<223> Xaa at residue 1 is Gln or pyro-Glu; Xaa at residue 2 is Trp (D or L), neo-Trp or halo-Trp (D or L); Xaa at residue 6 is Lys, N-methyl-Lys, N,N,-dimethyl-Lys or N,N,N-trimethyl-Lys.

<220>
<221> PEPTIDE
<222> (7)
<223> Xaa at residue 7 is Glu or gamma-carboxy-Glu.

<400> 9
Xaa Gly Xaa Cys Cys Xaa Xaa Asn Ile Arg Cys Cys Val
1 5 10

<210> 10
<211> 15
<212> PRT
<213> Conus textile

<220>
<221> PEPTIDE
<222> (1)..(13)
<223> Xaa at residues 1 and 4 is Glu or gamma-carboxy-Glu; Xaa at residue 7 is Trp (D or L), neo-Trp or halo-Trp (D or L); Xaa at residue 13 is Pro or hydroxy-Pro.

<400> 10
Xaa Cys Cys Xaa Asp Gly Xaa Cys Cys Thr Ala Ala Xaa Leu Thr
1 5 10 15

<210> 11
<211> 15
<212> PRT
<213> Conus textile

<220>
<221> PEPTIDE
<222> (4)..(13)
<223> ; Xaa at residue 4 is Glu or gamma-carboxy-Glu; Xaa at residue 7 is Trp (D or L) neo-Trp or halo-Trp (D or L); Xaa at residue 13 is Pro or hydroxy-Pro.

<400> 11
Gly Cys Cys Xaa Asp Gly Xaa Cys Cys Thr Ala Ala Xaa Leu Thr
1 5 10 15

<210> 12
<211> 20
<212> PRT
<213> Conus marmoreus

<220>
<221> PEPTIDE
<222> (14)..(17)
<223> Xaa at residue 14 and 17 is Glu or gamma-carboxy-Glu; Xaa at residue 16 is Lys, N-methyl-Lys, N,N-dimethyl-Lys or N,N,N-trimethyl-Lys.

<400> 12
Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser Arg Phe Xaa Ile Xaa
1 5 10 15

Xaa Asn Asp Phe
20

<210> 13
<211> 10

<212> PRT
<213> Conus marmoreus

<400> 13
Asn Ala Cys Cys Ile Val Arg Gln Cys Cys
1 5 10

<210> 14
<211> 11
<212> PRT
<213> Conus marmoreus

<400> 14
Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser
1 5 10

<210> 15
<211> 11
<212> PRT
<213> Conus caracteristicus

<220>
<221> PEPTIDE
<222> (3)
<223> Xaa at residue 3 is Pro or hydroxy-Pro.

<400> 15
Cys Cys Xaa Arg Arg Leu Ala Cys Cys Ile Ile
1 5 10

<210> 16
<211> 10
<212> PRT
<213> Conus caracteristicus

<220>
<221> PEPTIDE
<222> (3)..(6)
<223> Xaa at residue 3 and 6 is Pro or hydroxy-Pro; Xaa at residue 5 is Lys, N-methyl-Lys, N,N-dimethyl-Lys or N,N,N-trimethyl-Lys.

<400> 16
Cys Cys Xaa Asn Xaa Xaa Cys Cys Phe Ile
1 5 10

<210> 17
<211> 10
<212> PRT
<213> Conus quercinus

<400> 17
Gly Cys Cys Ala Met Leu Thr Cys Cys Val
1 5 10

<210> 18
<211> 13
<212> PRT
<213> Conus gloriamaris

<220>
<221> PEPTIDE
<222> (6)..(13)
<223> Xaa at residue 6 and 11 is Glu or
gamma-carboxy-Glu; Xaa at residues 8, 12 and 13 is
Trp (D or L), neo-Trp or halo-Trp (D or L).

<400> 18
Leu Cys Cys Val Thr Xaa Asp Xaa Cys Cys Xaa Xaa Xaa
1 5 10

<210> 19
<211> 11
<212> PRT
<213> Conus gloriamaris

<220>
<221> PEPTIDE
<222> (5)
<223> Xaa at residue 5 is Pro or hydroxy-Pro.

<400> 19
Val Cys Cys Arg Xaa Val Gln Asp Cys Cys Ser
1 5 10

<210> 20
<211> 554
<212> DNA
<213> Conus textile

<220>
<221> CDS
<222> (65)..(250)

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ggtactcaac gaacttcaag acacattctt ttcacctgga cacgggaagc tgactacaag 60

caga atg tgc tgt ctc cca gtg ttc gtc att ctt ctg ctg ctg att gca 109
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala
1 5 10 15

tct gca cct agc gtt gat gcc caa ccg aag acc aaa gat gat gtg ccc 157
Ser Ala Pro Ser Val Asp Ala Gln Pro Lys Thr Lys Asp Asp Val Pro
20 25 30

ctg gca cct ttg cac gat aat gca aag agt gca cta caa cat ttg aac 205
Leu Ala Pro Leu His Asp Asn Ala Lys Ser Ala Leu Gln His Leu Asn
35 40 45

caa cgc tgc tgc caa aca ttc tat tgg tgc tgt gtt caa ggg aaa 250
Gln Arg Cys Cys Gln Thr Phe Tyr Trp Cys Cys Val Gln Gly Lys
50 55 60

tgaatttgga tgagacccct gcgaactgtc catggatgtg agatttggaa agcagactgt 310

tcctttcgca cgtgttcgtg gaattttgaa tggtcgttaa caacacgctg ccacttgcaa 370

gctactatct ctctgtcctt tcatctgtgg aactggatga cctaacaact gaaatatcat 430

agaaattttt cagtgggtat acactatgac catgtagtca gtaattacat catttggacc 490

ttttgaaata tttttcaaaa tgttaagatt tttccccng gaaaggnctt ttgaagtaaa 550

tatt 554

<210> 21
<211> 62
<212> PRT
<213> Conus textile

<400> 21
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser
1 5 10 15

Ala Pro Ser Val Asp Ala Gln Pro Lys Thr Lys Asp Asp Val Pro Leu
20 25 30

Ala Pro Leu His Asp Asn Ala Lys Ser Ala Leu Gln His Leu Asn Gln
35 40 45

Arg Cys Cys Gln Thr Phe Tyr Trp Cys Cys Val Gln Gly Lys
50 55 60

<210> 22
<211> 416
<212> DNA
<213> Conus geographus

<220>
<221> CDS
<222> (1)..(183)

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Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Thr Ser
1 5 10 15

gca cct agc gtt gat gct cta ccg aag acc agg gat gat gtg ccc cta 96
Ala Pro Ser Val Asp Ala Leu Pro Lys Thr Arg Asp Asp Val Pro Leu
20 25 30

gca tct ttc cac ggt gga tat aat gca agg aga atc cta caa agg cgt 144
Ala Ser Phe His Gly Gly Tyr Asn Ala Arg Arg Ile Leu Gln Arg Arg
35 40 45

cag ggc tgg tgc tgc aaa gaa aat att gcg tgc tgt ata tagtggtaac 193
Gln Gly Trp Cys Cys Lys Glu Asn Ile Ala Cys Cys Ile
50 55 60

gggaaatgac tttggatgag acccctgcaa actgtccctg gatgtgaaat ttggaaagta 253

gactgttcct ttcgcgcgtg ttcgtggaat ttcaaatggt cgtcaacaac acactgctac 313

ttgcaaagct actatctctc tgtcctttca tctgtggaac tgggtgatct aacagctgaa 373

atgtcgcaga aatttttcaa ttggtctata ctatgaccat gta 416

<210> 23
<211> 61
<212> PRT
<213> Conus geographus

<400> 23
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Thr Ser

```
  1               5                  10                  15

Ala Pro Ser Val Asp Ala Leu Pro Lys Thr Arg Asp Asp Val Pro Leu
             20                  25                  30

Ala Ser Phe His Gly Gly Tyr Asn Ala Arg Arg Ile Leu Gln Arg Arg
         35                  40                  45

Gln Gly Trp Cys Cys Lys Glu Asn Ile Ala Cys Cys Ile
     50                  55                  60
```

<210> 24
<211> 413
<212> DNA
<213> Conus quercinus

<220>
<221> CDS
<222> (1)..(186)

<400> 24

```
atg cgc tgt gtc cca gtc ttc atc att ctt ctg ctg ctg agt cca tct   48
Met Arg Cys Val Pro Val Phe Ile Ile Leu Leu Leu Ser Pro Ser
  1               5                  10                  15

gca cct agc gtt gat gcc cat ccg atg acc aaa gat gat gtg ccc cag   96
Ala Pro Ser Val Asp Ala His Pro Met Thr Lys Asp Asp Val Pro Gln
             20                  25                  30

gca tca ttc cat gat gat gca aag cga acc cta caa gta cct tgg atg   144
Ala Ser Phe His Asp Asp Ala Lys Arg Thr Leu Gln Val Pro Trp Met
         35                  40                  45

aaa cgc ggg tgc tgc gca agg ttg act tgc tgc gtt gga cga           186
Lys Arg Gly Cys Cys Ala Arg Leu Thr Cys Cys Val Gly Arg
     50                  55                  60

taaagggaaa tgactttgga tgagacccct gcgaactgtc cctggatgtg aaatttggac 246

agcagactgc tcctttcgca cgtgttcgtg gaattttgaa tggtcgttaa caacacgctg 306

ccacttgcaa gctattatct ctctgtccct ttatctgtgg aactggataa tctaacaact 366

gaaatgtcat tgaaaatttt caatggatat atattatgat ccatata               413
```

<210> 25
<211> 62
<212> PRT
<213> Conus quercinus

<400> 25

```
Met Arg Cys Val Pro Val Phe Ile Ile Leu Leu Leu Ser Pro Ser
  1               5                  10                  15

Ala Pro Ser Val Asp Ala His Pro Met Thr Lys Asp Asp Val Pro Gln
             20                  25                  30

Ala Ser Phe His Asp Asp Ala Lys Arg Thr Leu Gln Val Pro Trp Met
         35                  40                  45

Lys Arg Gly Cys Cys Ala Arg Leu Thr Cys Cys Val Gly Arg
     50                  55                  60
```

<210> 26
<211> 435
<212> DNA
<213> Conus imperialis

<220>
<221> CDS
<222> (26)..(211)

<400> 26
aattcggaag ctgactacaa gcaga atg tac tgt ctc cca gtc ttc atc att 52
Met Tyr Cys Leu Pro Val Phe Ile Ile
1 5

ctt ctg ctg ctg att tca tct gca cct agc act cct ccc caa cca agg 100
Leu Leu Leu Leu Ile Ser Ser Ala Pro Ser Thr Pro Pro Gln Pro Arg
10 15 20 25

aac aaa gat cgt gtg cac ctg ata tct tta ctc gat aat cac aag caa 148
Asn Lys Asp Arg Val His Leu Ile Ser Leu Leu Asp Asn His Lys Gln
30 35 40

atc cta caa aga gat tgg aac agt tgc tgt ggg aaa aat cct ggt tgc 196
Ile Leu Gln Arg Asp Trp Asn Ser Cys Cys Gly Lys Asn Pro Gly Cys
45 50 55

tgt cct tgg gga aaa tgactttgga tgagacccct gcaaactgtc cctggatgtg 251
Cys Pro Trp Gly Lys
60

agatttggaa agcagaccgt ttgtggaatt ttgaatggtc gttaacaaca cgctgccact 311

tgcaagctac aatctctctg tcctttcatc tttggaactg gatgatcaaa caactgaaat 371

gtcatagaaa tttttcaatg ggtatacaat atgtgggcat ttagtcagta attacatcat 431

ttgg 435

<210> 27
<211> 62
<212> PRT
<213> Conus imperialis

<400> 27
Met Tyr Cys Leu Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ser Ser
1 5 10 15

Ala Pro Ser Thr Pro Pro Gln Pro Arg Asn Lys Asp Arg Val His Leu
20 25 30

Ile Ser Leu Leu Asp Asn His Lys Gln Ile Leu Gln Arg Asp Trp Asn
35 40 45

Ser Cys Cys Gly Lys Asn Pro Gly Cys Cys Pro Trp Gly Lys
50 55 60

<210> 28
<211> 421
<212> DNA
<213> Conus geographus

<220>
<221> CDS
<222> (1)..(183)

<400> 28
atg tgc tgt ctc cca gtc ttc gtc att ctt ctg ttg ctg att aca tct 48
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Thr Ser
1 5 10 15

gca cct agc gtt gat gct cta ccg aag acc agg gat gat gtg ccc cta 96
Ala Pro Ser Val Asp Ala Leu Pro Lys Thr Arg Asp Asp Val Pro Leu
20 25 30

gca tct ttc cac ggt gga tat aat gca agg aga atc cta caa agg cgt 144
Ala Ser Phe His Gly Gly Tyr Asn Ala Arg Arg Ile Leu Gln Arg Arg
35 40 45

cag ggc tgg tgc tgc aaa gaa aat att gcg tgc tgt gta tagtggtaac 193
Gln Gly Trp Cys Cys Lys Glu Asn Ile Ala Cys Cys Val
50 55 60

gggaaatgac tttggatgag acccctgcaa actgtccctg gatgtgaaat ttggaaagta 253

gactgttcct ttcgcgcgtg ttcgtggaat ttcaaatggt cgtcaacaac acactgctac 313

ttgcaaagct actatctctc tgtcctttca tctgtggaac tgggtgatct aacagctgaa 373

atgtcgcaga aatttttcaa ttggtctata ctatgaccat gtagtcag 421

<210> 29
<211> 61
<212> PRT
<213> Conus geographus

<400> 29
Met Cys Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Thr Ser
1 5 10 15

Ala Pro Ser Val Asp Ala Leu Pro Lys Thr Arg Asp Asp Val Pro Leu
20 25 30

Ala Ser Phe His Gly Gly Tyr Asn Ala Arg Arg Ile Leu Gln Arg Arg
35 40 45

Gln Gly Trp Cys Cys Lys Glu Asn Ile Ala Cys Cys Val
50 55 60

<210> 30
<211> 431
<212> DNA
<213> Conus textile

<220>
<221> CDS
<222> (1)..(201)

<400> 30
atg cgc tgt ttc cca gtc ttc atc att ctt ctg ctg cta att gca tct 48
Met Arg Cys Phe Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser
1 5 10 15

gca cct tgc ttt gat gcc cga acg aag acc gat gat gat gtg ccc ctg 96

```
Ala Pro Cys Phe Asp Ala Arg Thr Lys Thr Asp Asp Asp Val Pro Leu
            20                  25                  30

tca tct ctc cgc gat aat cta aag cga acg ata cga aca cgc ctg aac   144
Ser Ser Leu Arg Asp Asn Leu Lys Arg Thr Ile Arg Thr Arg Leu Asn
         35                  40                  45

ata cgc gag tgc tgc gag gat gga tgg tgc tgt act gct gca ccc tta   192
Ile Arg Glu Cys Cys Glu Asp Gly Trp Cys Cys Thr Ala Ala Pro Leu
     50                  55                  60

aca ggt cgt tagggataaa ggaaaatggc tttggatgag acccctgcga           241
Thr Gly Arg
 65

attgtccctg gatgtgagat ttggaaagca gactgttcct ttcgcacgtg ttcgtggaat 301

ttcgaatggt cgttaacaac acgctgccac ttgcaagcca ccatctctct gtcctttcgt 361

atgtggaact gtatgatcta acaactgaaa tgtcagaaag ttttcagtgg gtatacacta 421

tgatcgtata                                                        431


<210> 31
<211> 67
<212> PRT
<213> Conus textile

<400> 31
Met Arg Cys Phe Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser
  1               5                  10                  15

Ala Pro Cys Phe Asp Ala Arg Thr Lys Thr Asp Asp Asp Val Pro Leu
            20                  25                  30

Ser Ser Leu Arg Asp Asn Leu Lys Arg Thr Ile Arg Thr Arg Leu Asn
         35                  40                  45

Ile Arg Glu Cys Cys Glu Asp Gly Trp Cys Cys Thr Ala Ala Pro Leu
     50                  55                  60

Thr Gly Arg
 65


<210> 32
<211> 441
<212> DNA
<213> Conus textile

<220>
<221> CDS
<222> (1)..(201)

<400> 32
atg cgc tgt ttc cca gtc ttc atc att ctt ctg ttg cta att gca tct   48
Met Arg Cys Phe Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser
  1               5                  10                  15

gca cct tgc ttt gat gcc cga acg aag acc gat gat gat gtg ccc ctg   96
Ala Pro Cys Phe Asp Ala Arg Thr Lys Thr Asp Asp Asp Val Pro Leu
            20                  25                  30
```

```
tca tct ctc cgc gat aat cta aag cga acg ata cga aca cgc ctg aac   144
Ser Ser Leu Arg Asp Asn Leu Lys Arg Thr Ile Arg Thr Arg Leu Asn
         35                  40                  45

ata cgc ggg tgc tgc gag gat gga tgg tgc tgt act gct gca ccc tta   192
Ile Arg Gly Cys Cys Glu Asp Gly Trp Cys Cys Thr Ala Ala Pro Leu
     50                  55                  60

aca ggt cgt tagggataaa ggaaaatggc tttggatgag acccctgcaa           241
Thr Gly Arg
 65

attgtccctg gatgtgagat ttggaaagca gactgttcct ttcgcacgtg ttcgtggaat 301

ttcgaatggt cgttaacaac acgctgccac ttgcaagcca ccatctctct gtcctttcgt 361

atgtggaact gtatgatcta acaactgaaa tgtcagaaag ttttcagtgg gtatacacta 421

tgatcgtata gtcagtaatt                                           441
```

<210> 33
<211> 67
<212> PRT
<213> Conus textile

<400> 33

```
Met Arg Cys Phe Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser
  1               5                  10                  15

Ala Pro Cys Phe Asp Ala Arg Thr Lys Thr Asp Asp Asp Val Pro Leu
             20                  25                  30

Ser Ser Leu Arg Asp Asn Leu Lys Arg Thr Ile Arg Thr Arg Leu Asn
         35                  40                  45

Ile Arg Gly Cys Cys Glu Asp Gly Trp Cys Cys Thr Ala Ala Pro Leu
     50                  55                  60

Thr Gly Arg
 65
```

<210> 34
<211> 416
<212> DNA
<213> Conus marmoreus

<220>
<221> CDS
<222> (1)..(210)

<400> 34

```
atg cgc tgc ctc cca gtc ttc gtc att ctt ctg ctg ctg att gca tct   48
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser
  1               5                  10                  15

gca cct agc gtt gat gcc cga ccg aag acc aaa gat gat atg ccc ctg   96
Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro Leu
             20                  25                  30

gca tct ttc cat gat aat gca aag cga atc ctg caa ata ctt cag gac   144
Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln Asp
         35                  40                  45
```

```
aga aat ggt tgc tgc aga gca gga gac tgc tgt tca cga ttt gag ata   192
Arg Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser Arg Phe Glu Ile
     50                  55                  60

aag gaa aat gac ttt gga tgagacccct gcaaactgtc cttggatgtg         240
Lys Glu Asn Asp Phe Gly
 65                  70

agatttggaa agcagactgt tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa 300

caacacgctg ccacttgcaa gctactatct ctctgtcctt ttgtctgtgg aactgtatga 360

tcaaacaact gaaatgtcat agaaattttt cagtgggtaa acactatgac catgta     416


<210> 35
<211> 70
<212> PRT
<213> Conus marmoreus

<400> 35
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser
  1               5                  10                  15

Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro Leu
            20                  25                  30

Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln Asp
         35                  40                  45

Arg Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser Arg Phe Glu Ile
     50                  55                  60

Lys Glu Asn Asp Phe Gly
 65                  70


<210> 36
<211> 487
<212> DNA
<213> Conus marmoreus

<220>
<221> CDS
<222> (3)..(179)

<400> 36
ga atg cgc tgc ctc cca gtc ttc gtc att ctt ctg ctg ctg att gca    47
   Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala
     1               5                  10                  15

tct gca cct agc gtt gat gcc cga ccg aag acc aaa gat gat atg ccc   95
Ser Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro
                20                  25                  30

ctg gca tct ttc cac gat aat gca aag cga atc ctg caa ata ctt cag   143
Leu Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln
            35                  40                  45

gac aga aat gct tgc tgc ata gta agg cag tgc tgt tgatgatttg        189
Asp Arg Asn Ala Cys Cys Ile Val Arg Gln Cys Cys
         50                  55

agataaagga aaatgacttt ggatgagacc cctgcaaact gtccctggat gtgagatttg 249
```

gaaagcagac tgttcctttc gcacgtgttc gtggaatttc gaatggtcgt taacaacacg 309

ctgccacttg caagctacta tctctctgtc ctttcatctg tggaactgta tgatcaaaca 369

actgaaatgt catagaaatt tttcagtggg taaacactat gatcatgtag tcagtaatta 429

catcatttgg aattccatca agcttatcga taccgtcgac ctcgaggggg ggcccggt 487

<210> 37
<211> 59
<212> PRT
<213> Conus marmoreus

<400> 37
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser
1 5 10 15

Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro Leu
20 25 30

Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln Asp
35 40 45

Arg Asn Ala Cys Cys Ile Val Arg Gln Cys Cys
50 55

<210> 38
<211> 370
<212> DNA
<213> Conus marmoreus

<220>
<221> CDS
<222> (1)..(180)

<400> 38
atg cgc tgc ctc cca gtc ttt gtc att ctt ctg ctg ctg att gca tct 48
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser
1 5 10 15

gca cct agc gtt gat gcc cga ccg aag acc aaa gat gat atg ccc ctg 96
Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro Leu
20 25 30

gca tct ttc cat gat aat gca aag cga atc ctg caa ata ctt cag gac 144
Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln Asp
35 40 45

aga aat ggt tgc tgc aga gca gga gac tgc tgt tca tgatttgaga 190
Arg Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser
50 55 60

taaagggaaa tgactttgga tgagacccct gcaaactgtc cttggatgtg agatttggaa 250

agcagactgt tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa caacacgctg 310

ccacttgcaa gctactatct ctctgtcctt tcatctgtgg aactgtatga tcaaacaact 370

<210> 39
<211> 60
<212> PRT

<213> Conus marmoreus

<400> 39
Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser
1 5 10 15

Ala Pro Ser Val Asp Ala Arg Pro Lys Thr Lys Asp Asp Met Pro Leu
20 25 30

Ala Ser Phe His Asp Asn Ala Lys Arg Ile Leu Gln Ile Leu Gln Asp
35 40 45

Arg Asn Gly Cys Cys Arg Ala Gly Asp Cys Cys Ser
50 55 60

<210> 40
<211> 413
<212> DNA
<213> Conus caracteristicus

<220>
<221> CDS
<222> (1)..(192)

<400> 40
atg cgc tgt ctc ccg gtc ttc atc att ctt ctg ctg ctg att gca tct 48
Met Arg Cys Leu Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser
1 5 10 15

gca cct ggc gtt gat gcc caa ccg aag acc aaa tat aat gcg ccc ctg 96
Ala Pro Gly Val Asp Ala Gln Pro Lys Thr Lys Tyr Asn Ala Pro Leu
20 25 30

aca tct ctc cac gat aat gca aag ggt ata cta caa gaa cat tgg aac 144
Thr Ser Leu His Asp Asn Ala Lys Gly Ile Leu Gln Glu His Trp Asn
35 40 45

aaa cgc tgc tgc ccc aga agg ctt gcc tgc tgt att ata gga cgg aaa 192
Lys Arg Cys Cys Pro Arg Arg Leu Ala Cys Cys Ile Ile Gly Arg Lys
50 55 60

tgaatgattt tgggtgagat ccctgcaaac tgtccctgga tttgaatttt ggaaagcaga 252

ctgttccttt cgcacgtgtt cgtggaattt cgaatggtcg ttaacaacac gctgccactt 312

gcaagctact atctctctgt ccttttttctc tgtgaaactg gatggtctaa caactgaaat 372

gtcatagaaa attttcaatg ggtatactct atgaccatct a 413

<210> 41
<211> 64
<212> PRT
<213> Conus caracteristicus

<400> 41
Met Arg Cys Leu Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser
1 5 10 15

Ala Pro Gly Val Asp Ala Gln Pro Lys Thr Lys Tyr Asn Ala Pro Leu
20 25 30

Thr Ser Leu His Asp Asn Ala Lys Gly Ile Leu Gln Glu His Trp Asn

```
         35                  40                  45

Lys Arg Cys Cys Pro Arg Arg Leu Ala Cys Cys Ile Ile Gly Arg Lys
     50                  55                  60


<210> 42
<211> 410
<212> DNA
<213> Conus caracteristicus

<220>
<221> CDS
<222> (1)..(189)

<400> 42
atg cgc tgt ctc cca gtc ttc atc att ctt ctg ctg ctg att gca tct   48
Met Arg Cys Leu Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser
  1               5                  10                  15

gca cct ggc gtt gat gcc caa ccg aag acc aaa tat gat gcg ccc ctg   96
Ala Pro Gly Val Asp Ala Gln Pro Lys Thr Lys Tyr Asp Ala Pro Leu
             20                  25                  30

aca tct ctc cac gat aat gca aag ggt ata cta caa gaa cat tgg aac   144
Thr Ser Leu His Asp Asn Ala Lys Gly Ile Leu Gln Glu His Trp Asn
         35                  40                  45

aaa cgc tgc tgc ccc aac aag cct tgc tgt ttt ata gga agg aaa       189
Lys Arg Cys Cys Pro Asn Lys Pro Cys Cys Phe Ile Gly Arg Lys
     50                  55                  60

tgaatgattt tgggtgagac ccctgcaaac tgtccctgga tttgaatttt ggaaagcaga 249

ctgttccttt cgcacgtgtt cgtggaattt cgaatggtcg ttaacaacac gctgccactt 309

gcaagctact atctctctgt ccttttttctc tgtgaaactg gatggtctaa caactgagat 369

gtcatagaaa attttcaatc ggtgtactct atgaccatct a                     410


<210> 43
<211> 63
<212> PRT
<213> Conus caracteristicus

<400> 43
Met Arg Cys Leu Pro Val Phe Ile Ile Leu Leu Leu Leu Ile Ala Ser
  1               5                  10                  15

Ala Pro Gly Val Asp Ala Gln Pro Lys Thr Lys Tyr Asp Ala Pro Leu
             20                  25                  30

Thr Ser Leu His Asp Asn Ala Lys Gly Ile Leu Gln Glu His Trp Asn
         35                  40                  45

Lys Arg Cys Cys Pro Asn Lys Pro Cys Cys Phe Ile Gly Arg Lys
     50                  55                  60


<210> 44
<211> 413
<212> DNA
<213> Conus quercinus
```

<220>
<221> CDS
<222> (1)..(186)

<400> 44
atg cgc tgt gtc cca gtc ttc atc att ctt ctg ctg ctg agt cca tct 48
Met Arg Cys Val Pro Val Phe Ile Ile Leu Leu Leu Leu Ser Pro Ser
1 5 10 15

gca cct agc gtt gat gcc cat ccg atg acc aaa gat gat gta ccc cag 96
Ala Pro Ser Val Asp Ala His Pro Met Thr Lys Asp Asp Val Pro Gln
20 25 30

gca tct ctc cat gat gat gca aag cga acc cta caa gta cct tgg atg 144
Ala Ser Leu His Asp Asp Ala Lys Arg Thr Leu Gln Val Pro Trp Met
35 40 45

aaa cgc ggg tgc tgc gca atg ttg act tgc tgc gtt gga cga 186
Lys Arg Gly Cys Cys Ala Met Leu Thr Cys Cys Val Gly Arg
50 55 60

taaagggaaa tgactttgga tgagacccct acgaactgtc cctggatgtg aaatttggac 246

agcagactgc tcctttcgca cgtgttcgtg gaatttcgaa tggtcgttaa caacacgctg 306

ccacttgcaa gctattatct ctctgtccct ttatctgtgg aactggataa tctaacaact 366

gaaacgtcat tgaaaatttt caatggatat atattatgat ccatata 413

<210> 45
<211> 62
<212> PRT
<213> Conus quercinus

<400> 45
Met Arg Cys Val Pro Val Phe Ile Ile Leu Leu Leu Leu Ser Pro Ser
1 5 10 15

Ala Pro Ser Val Asp Ala His Pro Met Thr Lys Asp Asp Val Pro Gln
20 25 30

Ala Ser Leu His Asp Asp Ala Lys Arg Thr Leu Gln Val Pro Trp Met
35 40 45

Lys Arg Gly Cys Cys Ala Met Leu Thr Cys Cys Val Gly Arg
50 55 60

<210> 46
<211> 735
<212> DNA
<213> Conus gloriamaris

<220>
<221> CDS
<222> (70)..(258)

<400> 46
gggcaggtac tcaacgaact tcaggacaca ttcttttcac ctggacacgg gaaactgact 60

ataagcaga atg cgc tac cta cca gtc ttc gtc att ctt ctg ctg ctg att 111
Met Arg Tyr Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile
1 5 10

```
gca tct ata cct agc gat act gtc caa ctg aag acc aaa gat gat atg   159
Ala Ser Ile Pro Ser Asp Thr Val Gln Leu Lys Thr Lys Asp Asp Met
 15                  20                  25                  30

ccc ctg gca tct ttc cac ggt aat gga aga cga atc ctg cga atg ctt   207
Pro Leu Ala Ser Phe His Gly Asn Gly Arg Arg Ile Leu Arg Met Leu
                35                  40                  45

tca aac aaa cgc tta tgc tgt gtc acc gag gat tgg tgc tgt gaa tgg   255
Ser Asn Lys Arg Leu Cys Cys Val Thr Glu Asp Trp Cys Cys Glu Trp
            50                  55                  60

tgg taaaggaaaa tgactttgga tgagacccct gcaaactgtt tctggatgtg        308
Trp

agatttggaa agcagactgt tctttcgcac gtattcgtga aatttcgaat ggtcgttaac 368

aacacgctgc cacttgcaag ctgctatctc tctgtctttt catctgtgga actgtatgat 428

ctaacaactg aaatgtcata gacatttttc attgggtata cactatgacc atgtagccag 488

taattacatc atttggacct tttggatatt tttcagtatg taagtgtgtt cccttaaaaa 548

gtcctttgta attatgtatt ttaanaattt angttttgca cataaattgt aaaacgctgt 608

cctttctgtt gntcctacat cantggtggg gaaaagnaaa atgtttggcc ntggtcaaat 668

ttaaataatn accctgccgt ttnaatgcng ttattantgg tattttnaac nttgnacggt 728

taaactt                                                          735
```

<210> 47
<211> 63
<212> PRT
<213> Conus gloriamaris

```
<400> 47
Met Arg Tyr Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala Ser
  1               5                  10                  15

Ile Pro Ser Asp Thr Val Gln Leu Lys Thr Lys Asp Asp Met Pro Leu
             20                  25                  30

Ala Ser Phe His Gly Asn Gly Arg Arg Ile Leu Arg Met Leu Ser Asn
         35                  40                  45

Lys Arg Leu Cys Cys Val Thr Glu Asp Trp Cys Cys Glu Trp Trp
     50                  55                  60
```

<210> 48
<211> 374
<212> DNA
<213> Conus gloriamaris

<220>
<221> CDS
<222> (3)..(188)

```
<400> 48
ga atg cgc tgt ctc cca gtc ttc gtc att ctt ctg ctg ctg att gca    47
   Met Arg Cys Leu Pro Val Phe Val Ile Leu Leu Leu Leu Ile Ala
     1               5                  10                  15
```

```
tct gca cct agc gtt gat gcc caa ccg aag acc aaa gat gat gtg ccc   95
Ser Ala Pro Ser Val Asp Ala Gln Pro Lys Thr Lys Asp Asp Val Pro
                20                  25                  30

ctg gca cct ttg cac gat aat ata agg agt act cta caa aca ctt cgg   143
Leu Ala Pro Leu His Asp Asn Ile Arg Ser Thr Leu Gln Thr Leu Arg
            35                  40                  45

aag aaa gtc tgc tgc cgc cca gtg cag gat tgc tgt tca ggg aaa       188
Lys Lys Val Cys Cys Arg Pro Val Gln Asp Cys Cys Ser Gly Lys
        50                  55                  60

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Ala Pro Leu His Asp Asn Ile Arg Ser Thr Leu Gln Thr Leu Arg Lys
        35                  40                  45

Lys Val Cys Cys Arg Pro Val Gln Asp Cys Cys Ser Gly Lys
    50                  55                  60
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/03021

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 15/12; A61K 38/00, 38/10; C07K 14/435, 14/00

US CL :514/13, 12, 2; 530/325, 324

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/13, 12, 2; 530/325, 324

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, EMBASE, CAS, conotoxins, peptides, Conus geographus and tulipa, pain, analgesic antagonist, receptor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRAY et al. Peptide Toxins from Conus *geographus* Venom. The Journal of Biological Chemistry. 25 May 1981, Vol. 256, No. 10, pages 4734-4740, see entire document.	1-37
A	NORTON et al. The Cystine Knot Structure of Ion Channel Toxins and Related Polypeptides. Toxicon. 1998, Vol. 36, No. 11, pages 1573-1583, see entire document.	1-37
A	SAVARIN et al. Three-Dimensional Structure of k-Conotoxin PVIIA, a Novel Potassium Channel-Blocking Toxin from Cone Snails. Biochemistry. 1998, Vol. 37, pages 5407-5416, see entire document.	1-37

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

31 MAY 2000

Date of mailing of the international search report

06 JUL 2000

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Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/03021

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,672,682 A (TERLAU et al.) 30 September 1997 (30.09.1997) column. 3, line 54- column 4, line 5 and column 15, line 28 - column 16, line 35.	1-37